VOLUME XXVII JULY, 1935 No. 3

DIGENETIC TREMATODES FROM SOME AUSTRALIAN FISHES

BY VIOLET WOOLCOCK

From the Department of Zoology, University of Melbourne

(With Plates VIII and IX and 6 Figures in the Text)

CONTENTS

PAGE

Introduction 309
Family Allocreadiidae
Helicometra tenuifolia n.sp. 310
Helicometra bassensis n.sp. 314
Dactylostomum gracile n.g. et n.sp. 315
Family Hemiuridae
Parahemiurus australis n.sp. 317
Erilepturus tiegsi n.g. et n.sp. 320
Family Gorgoderidae
Probolitrema philippi n.sp. 323
Probolitrema antarcticus n.sp. 325
Family Azygiidae
Otodistomum pristiophori (*Distomum pristiophori* Johnston) . . 330
References 330
Explanation of Plates 331

INTRODUCTION

VERY little work has been done on the systematic study of entozoic trematodes of Australian fishes. Possibly the earliest contribution to the subject was a work by Kreft (1871), "On Australian Entozoa." Other contributors to this branch of the subject are Wm Nicoll, S. J. Johnston, and T. Harvey Johnston, their studies being chiefly based on parasites of fishes from Queensland and New South Wales waters. So far as can be ascertained there has been as yet only one contribution to the literature on trematode parasites of fishes of Southern Australian waters, a paper by T. Harvey Johnston recently published.

In the present paper seven new forms are recorded parasitic in fish from this region. In addition, a form described by S. J. Johnston from New South Wales waters is now recorded from Southern Australian waters.

The worms comprise the following species:

From Teleost fishes:

Helicometra tenuifolia n.sp. from the intestine of *Helicolenus percoides* (Red Gurnet).

Helicometra bassensis n.sp. from the intestine of *Platycephalus bassensis* (Flathead).

Dactylostomum gracile n.g. et n.sp. from the stomach, pyloric caeca and intestine of *Myxus elongatus* (Sand Mullet).

Parahemiurus australis n.sp. from the intestine of *Myxus elongatus* (Sand Mullet).

Erilepturus tiegsi n.g. et n.sp. from the stomach of *Arripis trutta* (Salmon Trout).

From Elasmobranch fishes:

Probolitrema philippi n.sp. from the coelom of *Heterodontus philippi* (Port Jackson Shark).

Probolitrema antarcticus n.sp. from the coelom of *Pristiophorus cirratus* (Gummy Shark).

Otodistomum pristiophori S. J. Johnston from the coelom of *Pristiophorus cirratus* (Sawfish).

An account of the anatomy of each new species is given, together with a diagnosis of the new genera and some remarks on their affinities and systematic position.

In the preparation of mounted specimens alum cochineal staining after fixation with formalin was found to be very satisfactory, while equally good results were obtained with iron haematoxylin or Delafield's haematoxylin following on fixation with Bouin.

To Dr O. W. Tiegs, under whose direction this work was carried out, the writer is indebted for much valuable assistance and helpful criticism.

Family Allocreadiidae

Helicometra tenuifolia n.sp. (Pl. VIII, fig. 1)

Marine form, found in the intestine of *Helicolenus percoides* (Red Gurnet).

Locality: Port Philip Bay, Victoria.

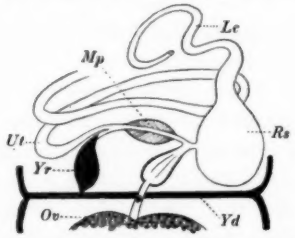
Incidence: Five out of the six fish examined were found to be infested, four having only one specimen each, the fifth host having about one dozen, mostly immature forms.

Described from living and stained specimens.

Length of formalinised specimens 5–7 mm.; breadth 1·25–1·5 mm. Post-acetabular portion of the body fairly broad, thin, and leaf-like, in the living animal showing a frilled edge; pre-acetabular region very narrow, attenuated. Integument devoid of cuticular spines. Beneath the thin and transparent cuticle of the ventral surface in living specimens may be seen numerous narrow finger-like structures, scattered irregularly in the parenchyma, chiefly in the anterior region of the body. They appear singly, are often branched, and vary in length from 10μ to about 1 mm. The internal end of each structure appears to terminate blindly, while the external usually becomes directed vertically to open on to the ventral surface by a minute pore. In some cases, however, the structures run transversely throughout their length, opening to the exterior on the lateral body margin. In appearance hyaline with a finely granular

content, they are not so clearly visible in fixed specimens. They exhibit no affinity for the stains used, Delafield's haematoxylin and alum cochineal, and no nuclei are demonstrable. In the living specimens refractile drops of an oily nature were seen to occur on the cuticular surface surrounding each pore. Although the actual secretion of the drops was not observed, it is presumed that the structures are of the nature of secretory organs.

A short prepharynx leads back from a slightly elongate anterior sucker, and communicates with a cylindrical muscular pharynx 0·2 mm. in length, The oesophagus varies from equal to twice the length of the pharynx, and the intestinal bifurcation occurs about half-way between the anterior and ventral suckers, the diverticula reaching almost to the posterior end of the body. The acetabulum, 0·5 mm. in diameter, is globular and situated at about one-quarter the body length from the anterior end. The ratio of the diameters of the suckers is 1 : 1. A short distance anterior to the acetabulum, on the ventral body surface, is to be seen a small, transverse, deep groove (Pl. VIII, fig. 1, *Vg*).



Text-fig. 1. *Helicometra tenuifolia.* Female reproductive organs. Shell gland omitted for clarity.

The excretory pore is dorsal, median, and subterminal, and unusually conspicuous. It passes to a long narrow cylindrical vesicle which extends to the posterior border of the ovary in some specimens, to half-way through the uterine area in others, before giving rise to two narrow undulating branches. These branches pass out like the arms of the typical Y-shaped excretory vesicle, taking an oblique course towards the ventral surface; but in addition to the usual prolongation of the limbs towards the anterior end of the body, in this case to the level of the pharynx, there is a backward prolongation to a short distance beyond the posterior testis. The terminations of the main lateral branches, anterior and posterior, are plainly visible in living specimens, owing to the flickering of a large flame cell at each blind end.

The median genital pore lies a short distance in front of the ventral groove. The cirrus sac is boomerang-shaped, extending from anterior to the ventral sucker, occasionally as far back as its posterior border. The basal half is filled with a large and slightly coiled vesicula seminalis, the distal half with a moderately long pars prostatica and a short but broad unarmed exsertile cirrus. The prostatic cells are exceedingly numerous and lie anterior and posterior to the pars prostatica (Pl. VIII, fig. 2). From the posterior end of

the vesicula seminalis the vasa deferentia pass backwards, close together for a short distance, then separating, one passing on either side of the ovary, the left passing to the anterior testis, the right to the posterior. The testes are tandem, entire, round to oval, and may or may not be contiguous. They are median in position and lie in the intercaecal space.

The ovary is median, directly in front of the anterior testis, slightly lobate, and more or less elongate transversely. From the anterior surface, a short, narrow duct projects into a wider ciliated chamber, which communicates with the large flask-shaped receptaculum seminis on the right, by a short duct from its distal end (Text-fig. 1). The ciliated section of the oviduct, the outer walls of which appear to be cuticularised, now gives place to a muscular propulsive portion. This leads into the ootype which receives a short duct from the yolk reservoir, before passing on as the uterus. The shell gland is exceptionally large, occupying the intercaecal space in the region between the ovary and the main portion of the uterus. This latter consists of about eight superimposed loops which lie between the ovary and acetabulum, and have the characteristic spiral arrangement of the genus. The metraterm is almost straight, and passes usually between the acetabulum and cirrus sac to the genital pore. A Laurer's canal is present and is directly continuous with the receptaculum seminis, opening on to the dorsal surface of the body. Movements of the female system were observed in living specimens but presented no unusual features.

The vitellaria are exceedingly well developed, the numerous and rather scattered medium-sized follicles extending from half-way up the uterine area to the posterior end of the body, and completely fill the post-testicular space. The vitelline ducts have the usual disposition, the transverse ducts uniting anterior to the ovary in a very conspicuous yolk reservoir, which lies usually to the left of the median line.

The eggs are provided with unipolar non-motile filaments, and measure $66–72 \times 21–27 \mu$. The filaments may reach a length seven times that of the ova. In the metraterm the eggs become separated and are possibly deposited singly. In the uterine coils, their arrangement, which has been described as "beads pendant from a supporting cord" completes the configuration of the uterus, peculiar to the genus.

No difficulty arises in assigning the species here described to its systematic position, the possession of filamented eggs excluding all genera of the Allocreadiidae but three. These are: (1) *Helicometra* Odhner (1902), (2) *Stenopera* (Manter, 1933), (3) *Helicometrina* (Linton, 1910). *Stenopera* differs from the species described here in the shape and great length of the cirrus sac, and in the far anterior position of the ventral sucker, these differences being of generic significance.

Members of the genus *Helicometrina* are characterised by the possession of two rows of testes, 5–9 in number, whereas the form just described has only two.

This leaves for consideration only the genus *Helicometra*. Many of the species of the genus *Helicometra* were originally placed in the genus *Allocreadium*. Wallin (1909), in a revision of the genus, states that "*Creadium*" was the actual name given to the genus by Looss (1900), but it was found that "*Creadion*" (Viellot, 1816) was the name of a genus of birds, and on account of the similarity of the terms, Looss changed the name to *Allocreadium*.

A number of the earlier species of *Allocreadium* were named by Rudolphi, but the meagre descriptions given by him made difficult their identification by later investigators, and consequently a number of synonyms arose. When Odhner examined Rudolphi's specimens in 1902, he placed *A. fasciatum*, *A. sinuatum*, and *A. pulchella* in a new genus, *Helicometra*, since he considered that the possession of eggs with long unipolar filaments was of generic significance. Of these three species *H. pulchella* was given as the type, and the diagnosis of the genus as described by Odhner (1902) is essentially as follows:

Allocreadiinae with ventral sucker at beginning of the second third of the body. Oesophagus of moderate length; intestinal bifurcation about the middle of the neck region. Genital pore median, below the intestinal bifurcation. Cirrus sac extending at most to the middle of the ventral sucker, filled chiefly by a coiled vesicula seminalis, followed by a weakly developed pars prostatica and short cirrus. Testes either median or oblique, behind one another. Ovary lobed. Vitelline follicles dorsal and ventral to the diverticula. Receptaculum seminis usually to the right and extended in longitudinal direction of body. Uterus more or less spirally coiled between ovary and ventral sucker. Eggs in a single row, large and yellow, and provided with unipolar filaments. The egg filament reaches a length ten times that of the egg.

There are six known species of the genus *Helicometra*, from which it remains to differentiate the species here described. Palombi (1929) has recently revised the genus, giving a complete account of the species, including morphology, variations and synonyms. He recognises five species. These are: *H. pulchella* (Rud.), *H. fasciata* (Rud.), *H. torta* (Linton), and *H. execta* (Linton). The sixth species is *H. plovmornini* (Issaitschikow, 1928). The paper by Issaitschikow in which it is described was not procurable here, but a brief description of its chief characteristics is given by Manter (1933).

The species *H. spirale* of Travassos (1928) need not be considered here, since *Helicometra* is undoubtedly meant for *Helicotrema*, a genus of the Harmostomidae.

The proposed new species *H. tenuifolia* differs from *H. execta*, *H. fasciata*, *H. sinuata*, and *H. pulchella*, in the extent of the vitellaria. In addition it differs from *H. execta* in the number of testes, and from *H. pulchella* in the topographical relation of the testes to one another. It agrees with *H. torta* in that the vitellaria do not extend anterior to the ventral sucker, but differs from it and *H. plovmornini* in its characteristic alignment of the vitelline

follicles. Possibly, however, the most obvious diagnostic feature of the new species is the presence of the "secretory tubules," the exact nature of which has not yet been ascertained.

Helicometra bassensis n.sp. (Pl. VIII, fig. 3)

Marine form, parasitic in the intestine of *Platycephalus bassensis* (Flathead).

Locality: Port Philip Bay, Victoria.

Incidence: Three Flathead, of six examined, each contained two specimens.

Described from living and stained specimens.

Length of formalinised specimens 2·5–3·5 mm.; breadth 0·5–0·85 mm. Cuticle smooth, unspined. Post-acetabular region of the body thin, in life showing a slightly frilled border. The ventral sucker, 0·28 mm. in diameter, is situated at approximately one-quarter the body length from the anterior end, and is twice the diameter of the anterior sucker. The prepharynx is very small and inconspicuous, the pharynx muscular, nearly globular, but breadth slightly greater than length, and the oesophagus about as long or a little longer than the pharynx (0·14 mm.). The intestinal bifurcation occurs well in front of the ventral sucker, and the diverticula are straight and unbranched, and comparatively wider than in the preceding species. There is no ventral groove in this form.

The genital pore is median and lies on the ventral surface a short distance behind the pharynx. The cirrus sac extends from close behind the genital pore to slightly beyond the border of the acetabulum, passing on the left. The distal portion containing a well-developed unarmed exsertile cirrus, and a moderately short pars prostatica, is slightly narrower than the basal portion which contains the large and slightly convoluted vesicula seminalis. The prostatic cells are fairly numerous and extend anterior and posterior to the pars prostatica (Pl. VIII, fig. 4). The testes, two in number, are contiguous, and situated in the posterior half of the body. They are median, tandem, slightly lobate or entire, and transversely fill the intercaecal space.

The ovary is elongate transversely, and lies immediately anterior to the testes. It may be entire, but is usually slightly lobate, the lobes being confined to the anterior border.

There is a very large flask-shaped receptaculum seminis, with which Laurer's canal is directly connected as in the other species.

The uterus consists of eight or nine loops, superimposed spirally in the manner peculiar to the genus. The metraterm usually passes on the right side of the acetabulum to the genital pore.

The vitellaria have the disposition characteristic of the Allocreadiidae. The follicles, which lie very close together, in this species extend laterally from the posterior border of the ventral sucker to the posterior end of the body, pass around the posterior ends of the diverticula and completely fill the post-testicular space. The conspicuous yolk reservoir is situated anterior to the ovary, slightly to the left of the median line.

The eggs measure 63–66 × 24–27 μ. Their unipolar filaments may reach a length of five times that of the egg.

Of the known species of the genus *Helicometra*, the new species lies nearest to *H. plovmornini* and to *H. torta*. It differs from *H. plovmornini* in the relative sizes of the suckers, and so far as can be ascertained without access to Issaitschikow's paper, it differs also in the arrangement of the vitelline follicles. From *H. torta* it is differentiated by the greater extent of the vitelline glands which reach as far forwards as the ventral sucker. No reference to the relative distribution of the follicles in *H. torta* has been seen, but judging by illustrations of specimens of it, the follicles lie scattered freely in the parenchyma, distributed similarly to those in *H. tenuifolia*. In *H. bassensis* the close proximity of the individual follicles gives to the vitelline area a rather compact appearance, which is a specific character.

Dactylostomum gracile n.g. et n.sp. (Pl. VIII, fig. 5)

Marine form, found in the stomach, pyloric caeca, and intestine of *Myxus elongatus* (Sand Mullet).

Locality: Port Philip Bay, Victoria.

Incidence: Nearly all fishes examined were found to be infested with a number of these parasites.

The following description is based on observations of living material in addition to stained specimens.

Size of formalinised specimens very variable; length extending from 2·5 to 5 mm., with an average length of 4 mm.; breadth 0·35–0·65 mm.; average breadth 0·5 mm. Integument smooth, without spines. Body flattened dorso-ventrally, post-acetabular region with parallel sides and rounded hinder end; pre-acetabular region narrower than post-, tapering anteriorly, but not sharply demarcated.

The terminal anterior sucker is small, elongate rather than spherical, and poorly developed. The acetabulum, located on a short thick pedicel at one-fifth the body length from the anterior end, is, on the other hand, very large, extremely well developed, and very characteristic. Seen from the ventral aspect it is median in position and not at all conspicuous. In side view, however, when fully extended, the projecting portion with its six tentacle-like outgrowths surrounding a central raised more or less hemispherical mass, gives the structure an appearance not unlike that of a coelenterate polyp. The hemispherical protuberance alone may be retracted, when it becomes cone-shaped, or, it may be retracted in conjunction with the outgrowths, in which case the acetabulum appears as a normal sucker with a very narrow transverse aperture. The ratio of the diameters of the anterior and ventral suckers is approximately 1 : 3.

The alimentary system presents a somewhat unusual feature among distomatous forms, in that the distal ends of the narrow unbranched intestinal

diverticula unite in an arch towards the posterior end of the body. There is no anus as is found in certain related forms. The intestinal bifurcation occurs slightly anterior to the acetabulum, and is preceded by an oesophagus varying in length from 0·1 to 0·2 mm., a poorly developed subcylindrical pharynx, and a short prepharynx.

The terminal excretory pore passes to a narrow median vesicle which reaches to the level of the posterior border of the ovary before giving off two main branches. These branches take a slanting course to the sides of the body, and then proceed towards the anterior sucker, at the sides of which they end blindly. In their passage they give off lateral branches of still narrower calibre, which from opposite sides unite beneath the acetabulum and pharynx.

The common genital pore is occasionally median, situated on the ventral surface immediately behind the pharynx, but usually occurs slightly displaced to the left. The testes, two in number, are entire, subspherical, and variable in size, lying tandem about the middle of the body, and may or may not be contiguous. The vasa deferentia unite at a variable distance posterior to the acetabulum, and immediately enter a simple, elongate, sac-like vesicula seminalis. There is no definite prostatic region, but investing the anterior end of the vesicula seminalis are to be found a few gland-like cells, grouped together, and these may possibly be prostatic in function. There is no cirrus sac, and the long ductus ejaculatorius is poorly developed. The terminal part of the ductus possibly functions as an exsertile cirrus, for in two specimens it was seen to be extruded beyond the genital pore.

The ovary, which is considerably smaller than the testes, and situated anterior to them, is slightly lobate or entire. A Laurer's canal is present but no receptaculum seminis. The ootype, after receiving the narrow anteriorly directed oviduct, and a short median yolk duct, enlarges considerably to form the uterus, the coils of which contain numerous sperms in addition to the eggs, and are confined to the region between ovary and acetabulum. The unicellular glands which together constitute the small shell gland are few in number and not surrounded by any common investing membrane.

The vitelline follicles are moderately large and numerous, but scattered, and extend in the sides of the body from slightly anterior to the acetabulum to the posterior end of the body. The follicles of either side are arranged in a more or less double row, with the longitudinal yolk duct lying in between. Behind the testes the rows are confluent. The transverse yolk ducts unite anterior to the ovary in a conspicuous yolk reservoir which is a permanent structure though variable in size and shape.

The eggs are large, measuring from 70 to 80μ by 50 to 60μ. They are few in number and without filaments.

Among the known Distomatous Trematodes, few forms have the distal ends of the intestinal diverticula uniting posteriorly. Pratt (1902), in his "Synopsis of North American Invertebrates", records its occurrence in three genera in the subfamily Syncoelinae. These are *Progonus* (Looss), *Otiotrema*

(Setti), and *Syncoelium* (Looss), but apart from this one peculiarity of the intestinal diverticula, they have little in common with the trematode just described, the affinities of which lie undoubtedly with the Allocreadiidae, and in particular with the Coitocoecinae, the absence of an anus excluding a direct relationship with the genera *Opecoelus* and *Opegaster* assigned by Ozaki (1925) to the family Opecoelidae.

The subfamily Coitocoecinae was comparatively recently made by Poche (1925) for the genus *Coitocoecum* described by Nicoll (1915). While having many features in common with the Allocreadiidae, Nicoll considered that *Coitocoecum* could not be included in that family because of its lack of a cirrus sac, and because of the union of the intestinal diverticula posteriorly, but he did not discuss its probable systematic position.

According to Poche the absence of a cirrus sac, the union of the diverticula, and even the absence of the receptaculum seminis, do not justify the exclusion of *Coitocoecum* from the Allocreadiidae, but since it does not fit into any of the known subfamilies, he has made a new subfamily for it. Ozaki (1929), evidently unaware of Poche's "Das System der Platodaria," has created a separate family for Nicoll's species of *Coitocoecum* and four species described by himself, viz. *C. orthorchis*, *C. diplobulbosum*, *C. unibulbosum* and *C. plagiorchis*.

Dactylostomum agrees with the genus *Coitocoecum* in those features which are Allocreadiid in nature; in addition it agrees with *C. gymnophallum* (Nicoll) in the absence of a cirrus sac, and with all five species in the absence of a receptaculum seminis, and in the union posteriorly of the intestinal diverticula. *Dactylostomum* differs chiefly from the members of the genus *Coitocoecum* in the peculiar nature of its acetabulum. And since this difference is considered to be of generic significance, a new genus has been made for it, which, following the classification of Poche, is diagnosed as follows:

Coitocoecinae of somewhat elongate body form; ventral sucker larger than oral sucker and provided with finger-like outgrowths; intestinal diverticula narrow uniting at posterior ends; anus lacking; genital pore anterior to intestinal bifurcation, median or displaced to the left; no cirrus sac; testes posterior to ovary, tandem, median, entire; vitellaria lateral, confluent behind testes, extending beyond acetabulum; yolk reservoir anterior to ovary; Laurer's canal present, but no receptaculum seminis; uterus with only few coils, and these between ovary and ventral sucker; eggs without filaments. Intestinal parasites of marine fishes. Type species: *Dactylostomum gracile*.

Family HEMIURIDAE

Parahemiurus australis n.sp. (Pl. VIII, fig. 6)

Marine form found in the intestine of *Myxus elongatus* (Sand Mullet).

Locality: Port Philip Bay, Victoria.

Incidence: Only comparatively few specimens were obtained from each of

the host fish examined, but with few exceptions all were infested with this parasite.

Described from living and stained specimens.

Total length of formalinised specimens 1·5–3·5 mm.; maximum breadth (at level of vitellaria) 0·44–0·46 mm. Small, slender, cylindrical worms, with retractile tail appendage (ecsoma) varying from one-fourth to one-fifth total body length. Cuticle with the plications, characteristic of the Hemiurinae, sharply defined, and not extending beyond the soma. The oral sucker is small and more or less globular, while the ventral 0·32–0·4 mm. in diameter, also globular, is situated about one-sixth the body length from the anterior end. The ratio of the diameters of the suckers is 1 : 2. A prepharynx is lacking, and the small spherical pharynx leads into a narrow oesophagus which bifurcates about half-way between the oral and ventral suckers to form the intestinal diverticula. Retaining the calibre of the oesophagus for a short distance, each diverticulum then dilates to form a more or less ovoid chamber, after which, becoming narrow again, the diverticula pass backwards, in extended specimens descending into the appendix for the greater part of its length. The walls of the diverticula are crenated throughout their length.

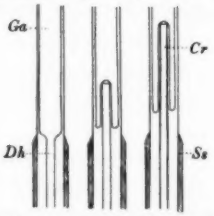
The main excretory vessels consist of a narrow cylindrical vesicle passing from the terminal excretory pore to the anterior border of the vesicula seminalis; and two branches of very narrow calibre, derivatives of the median vesicle, which pass around the acetabulum and forwards to the sides of the anterior sucker. Lateral branches from these arms pass transversely towards the median line, one pair uniting behind the pharynx.

The testes are post-acetabular, two in number, oval, median, and tandem, lying close together about the middle of the body length. The vasa deferentia are extremely short, and pass transversely to the left to the vesicula seminalis, a very conspicuous structure on account of its large size and strongly muscular walls. Undivided, and in form elongate with sides nearly parallel, the vesicula seminalis takes a slanting course, so that while its basal end lies lateral to the testes, its anterior end lies on the median line, a short distance posterior to the ventral sucker. The pars prostatica, which is moderately long and winding, is also post-acetabular, and together with the vesicula seminalis lies outside the long, muscular, cylindrical structure, to which the term "sinus sac" as applied by Manter (1926) is more appropriate than that of "cirrus sac" following the terminology of Looss (1907*b*). At a point just anterior to the pars prostatica, the male conducting tube uniting with the female, gives rise to the long, narrow ductus hermaphroditicus or genital sinus, which is surrounded by the sinus sac for the greater part of its length. The portion of the hermaphrodite duct included in the sinus sac functions here as the male copulatory organ, while the remaining portion, that is, the portion of the duct lying between the sinus sac and the genital pore, enlarges to form a short, weakly muscular genital atrium.

Movements of the terminal portions of the male genital organs were to be

clearly observed in the living material. The conversion of the ductus hermaphroditicus to a cirrus consists of an everting movement involving the wall of the atrium as well as that of the ductus itself, and resulting in the gradually increasing projection of the terminal portion of the ductus into the atrium as shown in Text-fig. 2.

In the female genital system, the ovary is median, ovoid, and post-testicular, lying at about the beginning of the third quarter of the body length. The vitellaria lie directly behind the ovary and consist of two compact lobed masses. The amount of lobulation of each mass in different specimens appears to vary only slightly. A small rounded receptaculum seminis is present, but neither in living nor stained specimens was it possible to make out its exact relations with the vitellaria or oviduct. The uterus is voluminous, and takes a course similar to that found in most species of the genus *Hemiurus*. Passing at first posteriorly along the ventral side, and descending into the appendix, it is then directed forwards towards the acetabulum, running on the dorsal side, winding round about the various organs, and making numerous small more or less transverse coils in its passage. At a point just anterior to the



Text-fig. 2. *Parahemiurus australis*. Terminal genital organs; to illustrate mechanism of eversion of cirrus.

pars prostatica it unites with the male duct to form the ductus hermaphroditicus. The exceedingly numerous eggs are very small, measuring $10 \times 18 \mu$.

In considering the systematic position of the species here described, three genera must be considered. These are (1) *Aphanurus* (Looss), (2) *Hemiurus* (Rud.), and (3) *Parahemiurus* (Vaz and Pereira, 1930). While agreeing with *Aphanurus* in its possession of an undivided seminal vesicle, the new form differs in having a distinct caudal appendix, and as this latter point is considered to be of generic significance, the genus *Aphanurus* (Looss, 1907*a*) is excluded.

From the known species of *Hemiurus*, with the exception of *H. merus* (Linton) and an unnamed species of Manter (1934), it differs in the condition of the seminal vesicle, which is here definitely undivided. The question has arisen as to whether this point is of generic significance. Manter (1934) says that there is some doubt as to whether the genus *Parahemiurus*, distinguished by the undivided seminal vesicle, should be considered as distinct from *Hemiurus*. Were there only a single species under consideration the creation of a separate genus might not appear justifiable, but the variation appearing in a number of different species points to the existence of a group variation, the members of which, sooner or later, could be expected to be split off from

the main group. For this reason the form here described has been assigned provisionally to the genus *Parahemiurus*.

A paper by Linton (1910) with the description of *Hemiurus merus* unfortunately was not available here. Since, however, Manter (1934) says that the description of *Parahemiurus parahemiurus* is very suggestive of *Hemiurus merus*, and the new form and the single species of *Parahemiurus* differ considerably, it may be presumed that it differs also from *Hemiurus merus*. Should the genus *Parahemiurus* be accepted, *Hemiurus merus* may be included in the genus *Parahemiurus*, either as a separate species, or with *P. parahemiurus* as a synonym, unless a further study reveals the vesicula seminalis to be inconspicuously divided. Differences, undoubtedly specific, which distinguish the species just described from *P. parahemiurus*, are to be found in the greater extent of the plications of the cuticle, in the presence of an elongate rather than pyriform vesicula seminalis, and lobed vitellaria compared with rounded.

Erilepturus tiegsi n.g. et n.sp. (Pl. IX, fig. 7)

Marine form, found in the stomach and intestine of *Arripis trutta* (Salmon Trout).

Locality: Port Philip Bay, Victoria.

Incidence: Of the six Salmon Trout investigated three had one or two specimens each.

Total length of formalinised specimens 4–6·5 mm.; maximum breadth (at level of ovary), 1·5–2 mm. Appendix (ecsoma) very variable, from one-fifth to one-eighth soma length. Body narrow, cylindrical. Cuticle not plicated as in *Parahemiurus*, but very finely ringed. This character, as also noted by Linton (1907) for a related genus, tends to disappear when the specimens have been under slight pressure for some time. Not to be confused with this faint ringing of the cuticle is the ringed appearance of these trematodes due to surface corrugations which involve both cuticle and muscles, and increase with the degree of contraction. The oral sucker is small, globular, and subterminal, the portion anterior to it appearing as a lip-like fold. A few scattered papillae, presumably sensory in function, are to be seen in this region, but are not visible in stained and mounted specimens. The ventral sucker, situated at about the middle of the body length, is large and globular, with a diameter approximately two and a half times that of the oral sucker. A prepharynx is lacking, and the small spherical pharynx 0·16 mm. in diameter, passes to a narrow, short oesophagus. The intestinal diverticula extend backwards into the appendix as in the previous form, but do not possess any specially demarcated portion, nor are their walls crenated.

The excretory pore is terminal, passing to the dilated terminal portion of the narrow excretory vesicle which bifurcates slightly posterior to the acetabulum into two undulating arms of yet narrower calibre. These arms pass on either side of the acetabulum proceeding anteriorly in the lateral regions of the

body, where at the level of the pharynx they curve inwards to meet behind the anterior sucker.

The common genital pore is median, situated immediately behind the pharynx, and passes to a large, ovoid, muscular atrium, from the basal end of which the ductus hermaphroditicus (genital sinus) leads off. Towards the genital pore, longitudinal and circular muscles are clearly to be observed in the wall of the atrium.

The testes, 0·35–0·4 mm. in diameter, are post-acetabular, two in number, oval and slightly elongated, and lie obliquely within the intercaecal space. The vasa deferentia are short, and unite just posterior to their entrance into the simple elongate sac-like vesicula seminalis. Thin walled, and appearing indistinctly tripartite in most though not all specimens, it is in the main post-acetabular, while the duct leading out from its anterior end is for the greater part of its length free from prostatic cells, the pars prostatica being confined to a moderately short section lying median, some distance anterior to the ventral sucker, and directly posterior to the ductus hermaphroditicus. The first part of this latter is slightly swollen, and surrounded by a strongly muscular sinus sac, the basal end of which is also swollen and bulb-like, the distal tapering anteriorly towards the atrium. On the wall of this latter section are to be observed, a short distance apart, two annular bands of muscle fibres, which function as sphincters during the process of egg-laying. The anterior termination of the majority of the muscle fibres of the sinus sac is on the wall of the ductus hermaphroditicus. This latter here also functions as a cirrus, but the wall of the atrium in this case apparently takes no part in its extrusion.

The ovary is median, oval, post-testicular, and slightly larger than the testes. The vitellaria lie directly behind the ovary, and consist of about six long irregular finger-like processes. A receptaculum seminis is present, but as in *Parahemiurus australis* its relation to other organs is not clear in either living or stained specimens. The uterus here is also voluminous and pursues a winding course similar to that of the previous genus, but does not enter the appendix. The union of male and female conducting tubes to give the ductus hermaphroditicus takes place directly anterior to the pars prostatica.

The eggs are numerous, small and oval, measuring $10 \times 22 \mu$. During the process of egg-laying the pre-acetabular portion of the body of the trematode undergoes alternating contractile and extensile movements. During extension a number of eggs, about 12–14, are drawn into the first portion of the ductus hermaphroditicus. Here they remain till the worm contracts, when a propulsive movement of this region sends them beyond the first muscle band, which now functioning as a sphincter immediately contracts and prevents their return. With a second extension a further batch of eggs is sucked in from the uterus to the ductus, while a further contraction followed by propulsive movements of the ductus sends the first eggs beyond the second band into the atrium, while the opening of the first sphincter allows their place to be taken by the second batch. Both muscle bands now contract, preventing any backward passage of

the eggs. During the time these movements were observed the atrium remained completely filled with eggs, from which it may be presumed that the number entering the atrium and leaving it were about equal. An accurate count of the trail of eggs left behind on the slide at each contraction was not possible. Considerable difficulty has been experienced in assigning this hemiurid to its systematic position, in part owing to the indefinite character of its cuticle, in part owing to its combining the characters of several genera. Concerning the ringing of the cuticle, regarded as a fundamental subfamily character among the Hemiuridae, the point for consideration has centred on the exact significance of the term "ringing". In the diagnosis of the subfamily Hemiurinae as cited by Looss (1907*b*) there is no doubt as to the nature of the transverse plications, for he says: "Haut im Vorderkörper mit scharf nach aussen vorspringenden Querleisten, die ihr im Profil das Aussehen einer Säge mit nach hinten gerichteten Zähnen geben." In the diagnosis of the subfamily Dinurinae however, only the term "quergeringelt" is given to describe the nature of the cuticle. The question arises as to whether "quergeringelt" (the meaning of which has already been extended to meet cases where the body rings tend to split up into scales) was originally meant only for the saw-like edge of the Hemiurinae, or meant to include a faint ringing such as described in the present form. Nicoll (1915) has evidently considered this latter to be the case, for in his description of *Ectenurus angusticauda* he states that "the cuticle is not markedly annulated, only traces of transverse wrinkles being present." That a feature, which, in the many genera and species of the Hemiuridae has proved so variable and inconstant—a feature too, which, as recorded earlier, has been shown to be affected by the slight pressure used during fixation—should be employed as a fundamental subfamily character does not appear at all satisfactory.

While the form here described appears to have features in common with both the Sterrhurinae and Lecithasterinae, it is felt that the cuticle cannot definitely be described as smooth, and on this account these subfamilies have been excluded. Possibly this hemiurid has more features in common with the Dinurinae than the Hemiurinae, but even then the former subfamily, as diagnosed by Looss, would have to be modified to receive it. The ecsoma here, though quite definite, is certainly not well developed. Looss, however, in the diagnosis of one of the genera of the Dinurinae, *Ectenurus*, modifies his statement "mit wohl-entwickeltem Abdomen," saying "Das Abdomen ist an sich wohlentwickelt, aber klein im Vergleich zu dem der *Dinuren*." He does not, however, indicate this in his diagnosis of the subfamily. Of the known genera of the Dinurinae, the affinities of the form just described appear to lie more closely with this genus *Ectenurus*, fully agreeing with the somewhat brief diagnosis. There are two species of the genus to be considered, *E. lepidus* (Looss) and *E. angusticauda* (Nicoll). In the structure of the terminal genital organs and the position of the acetabulum, the new form differs from both species. A further point of difference is to be found in the excretory system, the arms of

the excretory vesicle not meeting at the anterior end in *E. lepidus*, while they do so here. Nicoll does not mention the excretory system in his description of *E. angusticauda*. Any of these differences occurring separately would not justify the erection of a separate genus, but since all the peculiarities occur in the one species it is considered that the bounds of a logical generic limitation have been passed, and that the creation of a separate genus is justifiable.

The name *Erilepturus* is suggested, and from the above description is diagnosed as follows:

Small cylindrical Dinurinae, with short ecsoma. Cuticle of soma faintly ringed. Oral sucker simple, subterminal. Ventral sucker large, globular, at about the middle of the soma length. Ratio of diameters of suckers 2 : 5. Prepharynx lacking, oesophagus short, intestinal diverticula entering appendix. Arms of excretory vesicle meeting behind anterior sucker. Genital pore common, median. Genital atrium large, ovoid; sinus sac muscular, bulb-shaped. Testes post-acetabular, oval, entire, intercaecal, oblique. Vesicula seminalis tripartite, in main post-acetabular. Pars prostatica moderately short, the greater part of the male conducting tube free from prostatic cells. Ductus hermaphroditicus (genital sinus) longer than pars prostatica, functioning as cirrus. Ovary median, oval, and post-testicular. Vitellaria post-ovarian, narrow, irregular, flattened tubular processes. Receptaculum seminis present. Uterus voluminous, posterior extension not entering appendix. Union of male and female conducting tubes directly anterior to the pars prostatica. Eggs small, oval, numerous. Habitat: stomach of fishes. Type species: *Erilepturus tiegsi*. The species is named in honour of Dr O. W. Tiegs, Associate Professor of Zoology, University of Melbourne.

Family GORGODERIDAE

Probolitrema philippi n.sp. (Pl. IX, fig. 9)

Marine form, found in the coelom of *Heterodontus philippi* (Port Jackson Shark).

Locality: Port Philip Bay, Victoria.

Only a single specimen was obtained from the host, and no additional specimens were found in any of four other sharks examined.

Length of formalinised specimen 6 mm.; breadth 3·5 mm. Body flattened, oval, with abbreviated anterior end. The integument is smooth without spines of any kind. The oral sucker is elliptical and deep, while the ventral sucker, at approximately one-third the body length from the anterior end, is circular in outline, flat and shallow, and roughly twice the diameter of the oral sucker. A strongly developed and muscular pharynx leads directly out of the oral sucker, and passes to a short oesophagus furnished with a pair of small oesophageal glands at its junction with the pharynx. The intestinal limbs, which are wide and unbranched, do not quite reach the posterior end.

The excretory pore lies on the dorsal surface near the posterior end, and

leads into a tubular vesicle, the extent of which, however, it was not possible to make out.

The nervous system is conspicuous, the nerve cells being unusually so. The cerebral ganglia, with tracts of radiating nerve fibres, lie each side of the oesophagus connected by a typical dorsal commissure, while a pair of lateral cords run backwards outside the intestinal diverticula. No side branches could be observed. The central ends of the nerve fibres resemble the so-called "neuropilem" typical of the invertebrate nervous system (Text-fig. 3). Bipolar as well as unipolar nerve cells are quite prevalent.

There is no common genital pore as in the type species, the genital ducts opening separately to the exterior (Pl. IX, fig. 10). Their pores are median, lying just behind the bifurcation of the intestine on the ventral surface, male and female openings being close together, the male slightly anterior to the female. They do not occur in a sinus but in a depression covered with small

Text-fig. 3. *Probolitrema philippi*. Portion of dorsal commissure and lateral nerve cord of one side.

papillae, which appear to lie not only on the ventral surface but also in the overlying parenchyma, possibly to give added strength (Pl. IX, fig. 10).

The testes consist of numerous compact testicles (about 24 each side) varying in form from entire to deeply lobate, and lying ventral and lateral to the intestinal diverticula. The ducts from the separate follicles join up in groups of twos and threes, and enter a main longitudinal vas deferens on each side. The vasa deferentia unite at the level of the anterior border of the acetabulum and pass forwards to the vesicula seminalis which is short, tubular, and slightly coiled. There is no cirrus or cirrus sac, and the ductus ejaculatorius is poorly developed.

In the comparatively small and indistinctly trilobed ovary, granules possibly of a yolky nature, as well as ova, are to be observed. (E. von Ofenheim (1900) has reported the occurrence of yolk granules in *Anaporrhutum albidum* and *A. richiardii* (=*Probolitrema capense* Looss).) The anteriorly directed oviduct passes into a narrow tube, the ootype, which having received a small median yolk duct and a duct from the receptaculum seminis, runs more or less transversely through the small indistinctly lobed shell gland, and then enlarges to form the uterus. This latter is thrown into a series of close convolutions which, passing backwards, reach almost to the posterior end, and fill the space

between the intestinal diverticula. The uterus, at the level of the transverse yolk duct, is then directed forwards as a straight tube, folding on itself once again in the region of the acetabulum before finally reaching the genital pore.

The rounded receptaculum seminis lies slightly to the left side and is unusually large, being 0·75 mm. in diameter. There is no Laurer's canal.

The vitelline glands are exceedingly small and occur in two sets lying chiefly outside the intestinal diverticula. Each set consists of small rounded rather scattered follicles. The transverse yolk duct in *Probolitrema philippi* lies ventral to the ootype and makes connection with this latter by means of a very short median vertical duct.

The numerous eggs are small, chiefly oval, but with a few almost spherical scattered among the more elongate ones. The oval eggs measure from 48 to 60μ in length, by 36 to 42μ in breadth. The diameter of the spherical varies from 30 to 46μ.

Probolitrema antarcticus n.sp. (Pl. IX, fig. 11)

Marine form, found in the coelom of *Mustelus antarcticus* (Gummy Shark).

Locality: Port Philip Bay, Victoria.

Of four sharks examined two were infested and these heavily with this trematode, one having twenty specimens, the other fifty-eight.

Described from living specimens, whole mounts and sections.

Length of formalinised specimens 5–10·5 mm.; average length 7·5 mm.; Breadth 3–6·5 mm., with 5 mm. average breadth. Body flattened, oval, the anterior end, in life, capable of extreme elongation. The integument, as in its congener, is smooth, without spines of any kind. The oral sucker is similar in shape to that of *P. philippi*, but the acetabulum, while presenting no variation in position, in character is more strongly developed, and has a diameter at most only one and a half times that of the oral sucker. The cup-shaped pharynx, 0·5 mm. in transverse diameter, in this species also is strongly muscular, and there is no prepharynx. The oesophagus, however, in *P. antarcticus* is considerably longer, being at least twice the length of the pharynx. The intestinal diverticula, which are wide and unbranched as in the other species, may show a number of small sacculations. These, however, are by no means a constant feature, and examination of the living material shows that they increase in number and size with increased contraction of the individual.

The main excretory channels are best observed in living specimens. The excretory pore lies on the dorsal surface at the posterior end, and leads into a wide straight tubular vesicle which extends to the posterior border of the ovary. Here it divides into two branches of narrow calibre. These cross the transverse yolk duct passing obliquely towards the ventral surface, and reaching a position in front of the vitelline glands are directed forwards, ending blindly a short distance from the anterior end.

The nervous system presents the characteristic ganglia, dorsal commissure and lateral nerve cords as usually found in typical Digenea.

As in the previous species there is no common genital pore; the separate pores are similarly median, and here also the male opening is usually slightly anterior to the female, but neither sinus, depression, nor papillae are present. The region, however, is made conspicuous by the presence of numerous darkly staining epithelial cells in the parenchyma about the metraterm and ductus ejaculatorius (Pl. IX, fig. 12). The testes are of the same type as in the previous species, the testicles numbering from 24 to 30 each side, but here in general are rather more scattered. Usually they lie completely outside the intestinal diverticula, barely reaching to the posterior end of the latter. In rare cases, however, isolated testicles are to be found within the space bounded by the intestinal limbs, or beyond their posterior ends, or even anterior to the vitelline glands. The vasa deferentia uniting directly anterior to the acetabulum immediately enter a long narrow and slightly coiled vesicula seminalis, the terminal portion of which expands into a simple, more or less oval sac, sometimes incompletely divided into two. The first section of the vesicula seminalis has been termed the "deferente" by Monticelli in his description of the type, while the ducts from the testicles he terms "dottolini testicolari." In this species the presence of a valve just anterior to the junction of the ducts is a definite indication that the structure formed by their union is intended for storage of sperms, and hence here is considered a portion of the vesicula seminalis, and the ducts the vasa deferentia. The ductus ejaculatorius is better developed here than in *P. philippi*; the section directly continuous with the vesicula seminalis in some specimens appears dilated, possibly due to muscular contraction. The numerous darkly staining cells investing this basal section of the ductus correspond in position, and may also correspond in function with the prostatic gland cells of other species of *Probolitrema*, although no ducts were discernible. Nevertheless the presence of numerous villus-like processes projecting into the cavity of this section points to some specific function, whether prostatic or otherwise. There is no cirrus sac ("tasca del pene") as recorded by Monticelli for the type.

Though varying considerably in shape and size, the ovary is always distinctly lobed, three being the usual number of lobes. In individual cases, however, the number may increase to four or even five. Granules (presumably yolk granules) in the ovary are to be observed here as in *P. philippi*, but in this case the granules are considerably larger, and an examination of longitudinal horizontal sections shows that they occur in the cytoplasm of each ovum (Pl. IX, fig. 13). A thin-walled and narrow oviduct arises anteriorly from the ovary and after a short distance bends more or less at right angles passing transversely to the left, having then a somewhat greater diameter than that of the preceding section (Pl. IX, fig. 14). Directly anterior to the ovary and oviduct lies the large round and dorso-ventrally flattened receptaculum seminis 0·75–1 mm. in diameter. This vesicle makes connection with the oviduct by a short thick duct, the distal end of which may project temporarily into its cavity as a valve, while after the union of its basal end with the transverse

oviduct, this latter continues as a narrow but now ciliated tube which widens into a rounded ciliated chamber. A narrow ootype with flattened epithelium and muscular walls leads out from the chamber after it has received a small median yolk duct, and later widens to give rise to the uterus. Both ciliated chamber and first part of the ootype are surrounded by a large lobed shell gland which is enclosed in a definite membrane.

As in the previous species, there is no Laurer's canal.

Movements of the female system were observed in living material. The first part of the transverse oviduct appears to be the main propellant organ of the system. This section periodically fills up, rounds itself and then contracts, the contraction of its walls forcing the contents of the tube, ova with granules, and fluid, partly into the receptaculum seminis, partly into the ciliated tube. The receptaculum may now contain ova and granules in addition to numerous motile sperms. The presence of ova, and in large numbers, in a receptaculum for sperms is unexpected. No reference commenting on such an occurrence has been seen. Withdrawal of the valve-like projection brings about the re-entry of some of the receptacular contents into the oviduct, and with further contraction of the propulsive organ, the contents are swept into the ciliated section. The probability is that by now all ova reaching here, whether direct or from the receptaculum, are fertilised, for, in this region, live sperms occur in large numbers, and so ensure fertilisation of those ova not passing to the receptaculum. The periodic contractile movements continuing, the contents of the ciliated tube are propelled along its length, and now enter the ciliated chamber, meeting yolk cells reaching there by the median yolk duct. Partly due to contraction of the transverse yolk duct, and partly due to suction of fluid as it moves along the oviduct, the yolk finds its way from the transverse duct across the median duct. This latter functions as a temporary yolk reservoir, the walls stretching periodically to accommodate extra yolk cells, and collapsing on their removal. The rapid flickering of the cilia in the ciliated chamber brings about a rotatory movement of the contents, and it is conceivable that such a movement would ensure an equal distribution of the shell material over the egg contents.

The uterus, chiefly occupying a position between the intestinal diverticula posterior to the ovary as in *P. philippi*, is much narrower in diameter, and therefore in contrast appears less compact. When directed forwards towards the acetabulum and genital pore, a winding course replaces the straight tube of the other species, and the walls of the terminal portion become thickened and muscular to form the simple metraterm.

The vitellaria, comparatively small here also, are formed by two lateral branched glands, which, in the main lie external to the intestinal diverticula, and directly anterior to the testes. The branches resemble clumsy finger-like projections which frequently anastomose.

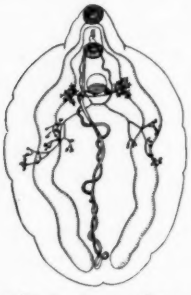
The eggs are numerous, small and ovoidal, and measure $36–45 \times 35–40 \mu$.

To determine the affinity of the two species last described, three genera of

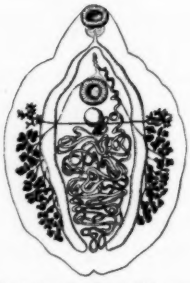
Anaporrhutinae must be considered: (1) *Anaporrhutum* (von Ofenheim, 1900, Text-fig. 4), (2) *Probolitrema* (Loos, 1902, Text-fig. 5), and (3) *Petalodistomum* (S. J. Johnston, 1913, Text-fig. 6). The position of the vitellaria mainly external to the intestinal diverticula, and the absence of intestinal branches are features which would seem to exclude direct relationship with *Petalodistomum*. The first two genera as defined by Looss are essentially as follows:

(1) *Anaporrhutum*: Large Anaporrhutinae with markedly broad hinder-body. Testes divided up into a number of somewhat rounded pieces, partly inside, partly outside the intestinal diverticula. Vitellaria lying internal to the intestinal diverticula. Habitat: Pericardium and coelom of sharks. Type species: *Anaporrhutum albidum* von Ofenheim.

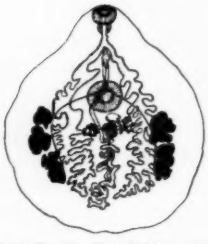
(2) *Probolitrema*: Large Anaporrhutinae with markedly broad hinder-body. Testes divided up into a number of somewhat rounded pieces, which together with the vitellaria lie completely outside the intestinal diverticula. Habitat: Coelom of sharks. Type species: *Probolitrema richiardii* (Lop.).



Text-fig. 4. *Anaporrhutum.*



Text-fig. 5. *Probolitrema.*



Text-fig. 6. *Petalodistomum.*

Both species described here appear to fall between *Anaporrhutum* and *Probolitrema* when considered only in the light of Looss' definition of the two genera, yet when other characters are taken into account they seem to be more closely related to *Probolitrema*. T. Harvey Johnston (1934), who has recently described some closely related forms, suggests an amendment to the generic diagnosis of *Probolitrema*, so as to include such forms as these, rather than erect a new genus. He proposes that the definition as applied to testicles and vitellaria should be extended to lying entirely or mainly outside the intestinal diverticula. In the case of the intestine the addition "though sacculations of the intestine may be present," in view of the findings here concerning their variability and impermanence, possibly may require further consideration.

On the other hand, since there exists a considerable difference in size between the type species and European forms generally, and the majority of the new Australian species, it is suggested here that Looss' definition of body size might be extended to "medium and large Anaporrhutinae."

Concerning the feature distinguishing the forms described here, *P. philippi* differs from the type in form of vitellaria, nature of the genital end-organs, in

the possession of a weakly developed ventral sucker, and possibly also in sucker ratio. Monticelli (1893), in his description of *Distomum richiardii* (=*Probolitrema richiardii*) does not give the sizes, or ratio of the diameters of the suckers, merely stating "la ventosa posteriore è assai piu grande dell' anteriore." If his illustration Fig. 51 on Pl. 5 is an accurate representation, then the ratio of the diameters of the suckers is not less than 1 : 3, compared with 1 : 2 in *P. philippi*. From all the other known species of *Probolitrema* the proposed new species differs in form of vitellaria and sucker ratio.

The second species of *Probolitrema*, described here as *P. antarcticus*, differs from the type, and from *P. capense* (Ofenheim), a closely related form, in the disposition of the vitellaria, elongate pre-acetabular portion of the body, form of uterus, and sucker ratio. It further differs from the type in its excretory system, and from *P. capense* in the form of the vitellaria. The term "Zweige" applied by E. von Ofenheim in his description of the branches of his species, would seem to imply a finer branching than is shown in *P. antarcticus*; *P. capense* was described by von Ofenheim as *Anaporrhutum richiardii*. Looss (1902), however, considered that the variation in the topography of the testes, and the different relationship of vitellaria to intestinal diverticula, were such as to exclude it from the genus *Anaporrhutum*, and placed it in the newly erected genus *Probolitrema*, with *Distomum richiardii* (Lop.) as the type, and it, *Anaporrhutum richiardii* (Ofenheim), as a further species, *Probolitrema capense*.

P. antarcticus differs considerably from *P. clelandi* (Johnston) and *P. simile* (Johnston), among other characters in sucker ratio, in its possession of a smooth cuticle, and in form of testes. With *P. rotundatum* (Johnston) the species under consideration has many features in common, and specific differences are not so marked. Obvious small differences include the long, narrow oesophagus, the large, conspicuous shell gland, and the smaller and rounder eggs. More significant, though at first sight less obvious differences, are to be found in the form of the uterus and vitellaria. Concerning the form of the uterus, there is no drawing to permit of making a direct comparison; but from Johnston's description, and from the fact that he does not comment on any variation, it is apparently the voluminous uterus of the type, and therefore quite unlike that of *P. antarcticus*.

When comparing the form of the vitellaria, that of the species here described and of *P. rotundatum* might easily be taken to be identical, were the comparison based solely on Johnston's description. But Johnston states later that his species differs from the type, among other things, in the form of the vitellaria, and his species must therefore be regarded as also differing in this respect from *P. antarcticus*, since the latter and the type are almost, if not quite, identical.

The above-mentioned differences appear to justify the form here described being considered as a separate species.

Family AZYGIIDAE

Otodistomum pristiophori (Distomum pristiophori) (Pl. IX, fig. 8)

Marine form, found in the coelom of *Pristiophorus cirratus* (Lath.) (Sawfish).

Locality: Port Philip Bay, Victoria.

Incidence: Only one specimen was obtained, though several host-fish were examined.

This trematode has been fully described as *Distomum pristiophori* by S. J. Johnston (1903), who obtained his specimens from the same fish, *Pristiophorus cirratus*, at Sydney. Odhner (1911) considers that Johnston's species is closely related to if not identical with *Otodistomum veliporum* (Crepl.), pointing out that the only difference in the Australian form is in the length of the egg and the thickness of the shell. Manter (1934) accepts this synonym without further comment. It is to be noted, however, that *Otodistomum veliporum* occurs in the stomach, *O. pristiophori* in the coelom. Johnston's statement of its occurrence in the coelom, doubted by Odhner, is here confirmed. Except for some minor points of difference, there is complete morphological agreement between *O. pristiophori* as described by Johnston, and the form found here.

The constrictions described by Johnston as occurring along the excretory canals are not confined to the canals in this case, but also occur in the excretory vesicle itself. There is no mention of a genital papilla, which here is large (0·4 mm. at base, 0·4 mm. length), and almost completely fills the basal end of the common genital atrium. The other slight differences are shown in the table of measurements below. The trematode is refigured here on account of the above-mentioned differences, and the doubt expressed as to its being a distinct species.

	Sydney specimen	Port Philip specimen
Length	25 mm.	14 mm.
Breadth	6 mm.	5·5 mm.
Oral sucker	1·5 mm.	1 mm.
Ventral sucker	2·5 mm.	1·54 mm.
Egg: Longitudinal diameter	77 μ	66–72 μ
Transverse diameter	62 μ	48–51 μ
Thickness of shell	8 μ	7·5 μ

REFERENCES

JOHNSTON, S. J. (1903). *Proc. Linn. Soc. N.S.W.* **27**, 326.

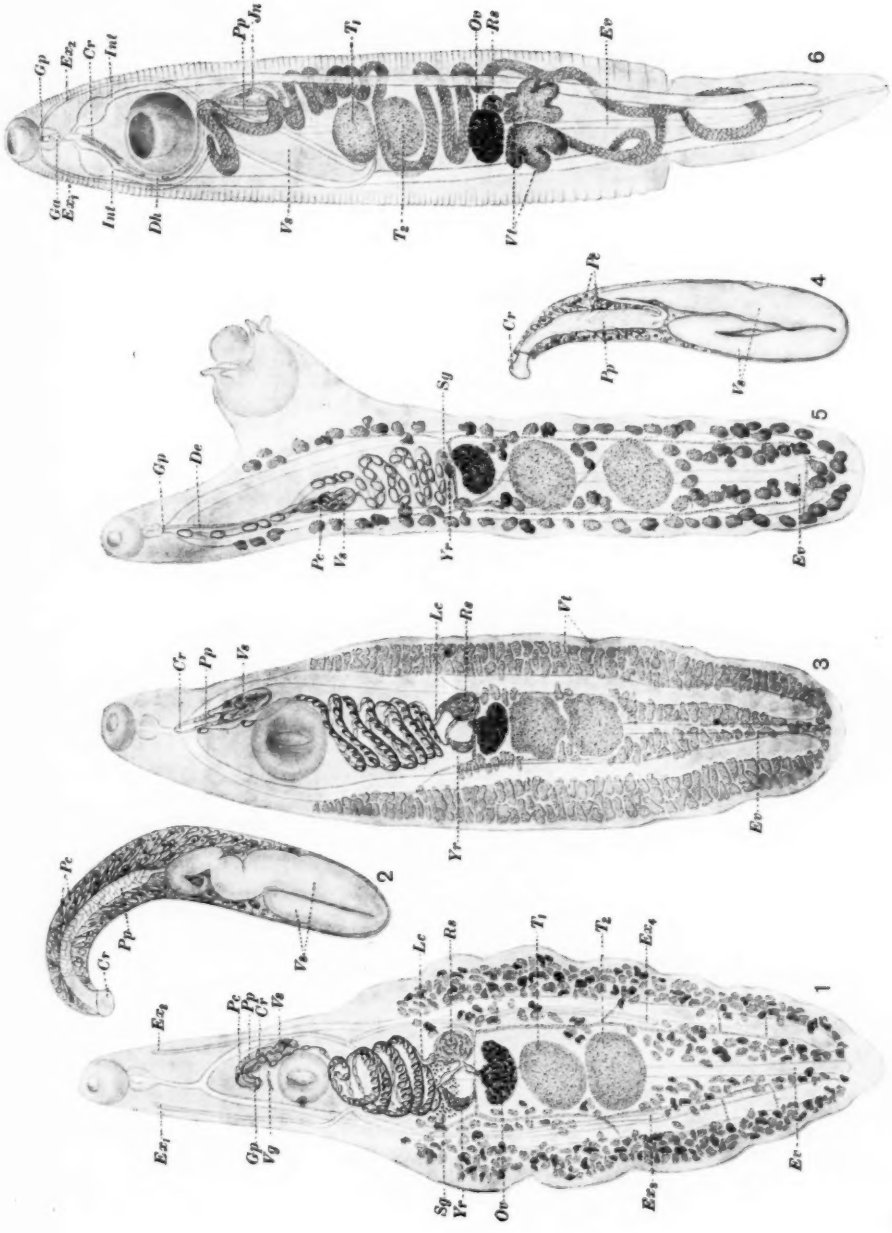
—— (1913). *Quart. J. micr. Sci.* **59**, 361.

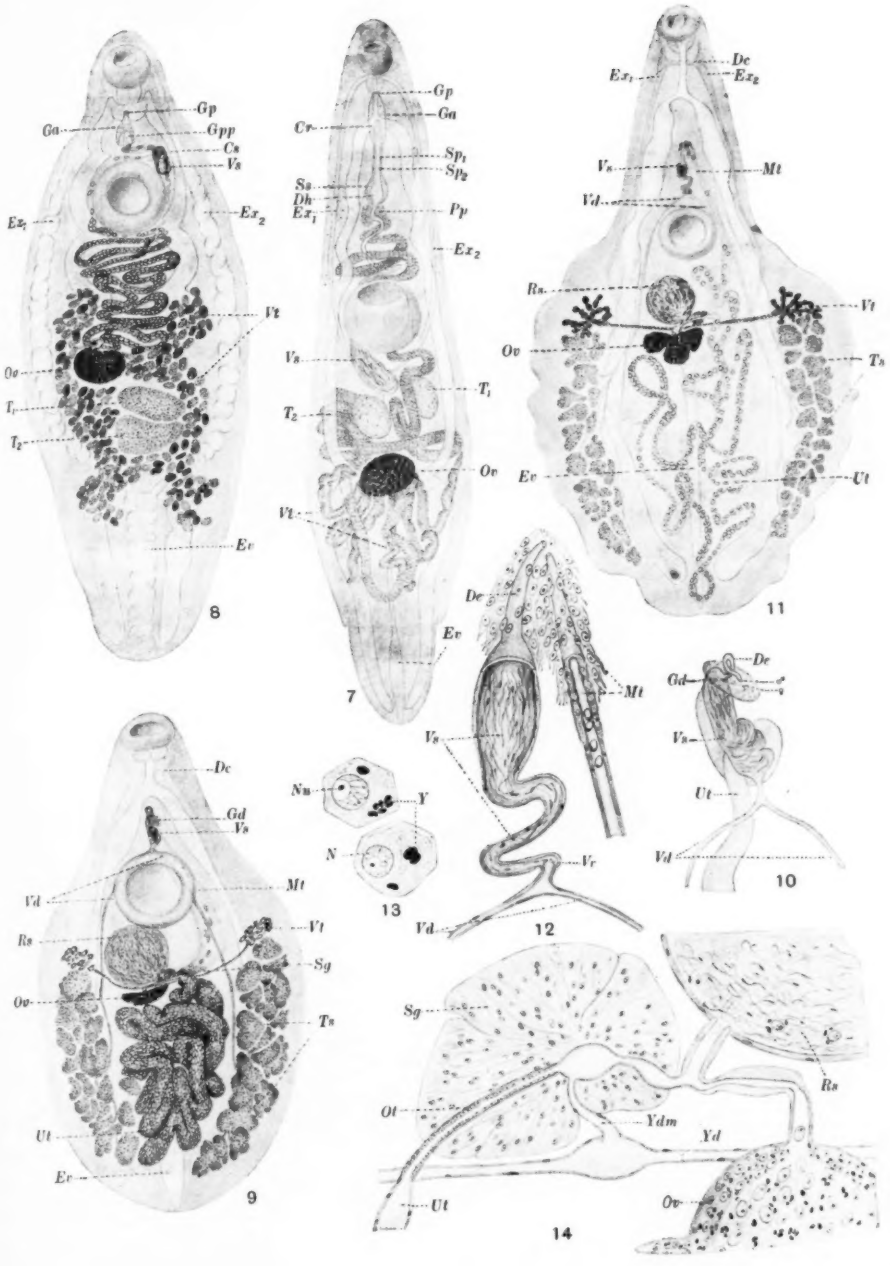
JOHNSTON, T. HARVEY (1934). *Aust. J. exp. Biol. med. Sci.* **12**, 28.

KREFT, G. (1871). *Trans. ent. Soc. N.S.W.* 206–32.

LINTON, E. (1907). *Proc. U.S. nat. Mus.* **33**, 85–126.

—— (1910). Publ. Carneg. Instn, No. 133, *Pap. Tortugas Lab.* **3**, 15–98.


Gp
Ex2
Cr
Int
Pp
Ju
T1
Ov
Rs
Ex
6
Ge
Ex1
Int
Dh
Ve
T2
Vl
Pe
Cr
Pp
Ve
4
Sg
Gp
De
5
Pe
Ve
Yr
Ex
Lc
Rs
Vl
Cr
Pp
Ve
3
Ex
Yr
Pe
Cr
Pp
Ve
2
Lc
Rs
T1
T2
Ex3
Ex4
Pe
Pp
Cr
Ve
1
Ex1
Gp
Vg
Sg
Yr
Ov
Ex3
Ex


Gp
Ga
Gpp
Cs
Vs
Ex1
Ex2
Vt
Ov
T1
T2
Ev
8
Gp
Ga
Cr
Sp1
Sp2
Sa
Dh
Ex1
Pp
Ex2
Vs
T1
T2
Ov
Vt
Ev
7
Ex1
De
Ex2
Vs
Mt
Vd
Rs
Vt
Ov
Ts
Ev
Ut
11
De
Mt
Vs
Vr
Vd
12
De
Gd
Vs
Ut
Vd
10
Nu
Y
N
13
Dc
Gd
Vs
Vd
Mt
Vt
Rs
Sg
Ov
Ts
Ut
Ev
9
Sg
Rs
Ot
Ydm
Yd
Ut
Ov
14

UNIV. OF MICH.

LOOSS, A. (1900). *Zool. Anz.* **23**, 601–8.
—— (1902). *Zool. Jb.* **16**, 411.
—— (1907 *a*). *Zool. Anz.* **31**, 585–620.
—— (1907 *b*). *Zool. Jb.* **26**, 63–180.
MANTER, H. W. (1926). *Illinois biol. Monogr.* **10**, No. 2.
—— (1933). Publ. Carneg. Instn, No. 435, *Pap. Tortugas Lab.* **28**, 169–80.
—— (1934). *Ibid.* **28**, 237–92.
MONTICELLI, F. S. (1893). *Zool. Jb.* Suppl. **3**.
NICOLL, W. (1915). *Parasitology*, **8**, 22–40.
ODHNER, T. (1902). *Zbl. Bakt.* **31**, 152–62.
—— (1911). *Zool. Anz.* **38**, 513.
OFENHEIM, E. VON (1900). Inaug. Dissert.
OZAKI, Y. (1925). *J. Parasitol.* **12**, 51–3.
—— (1929). *Annot. zool. jap.* **12**, 75–90.
PALOMBI, A. (1929). *Publ. Staz. zool. Napoli*, **9** (2), 237–345.
POCHE, F. (1925). *Arch. Naturgesch.* A, **91**, 1–451.
PRATT, H. S. (1902). *Amer. Nat.* **36**, 953–79.
TRAVASSOS, L. (1928). *Mem. Inst. Osw. Cruz*, **21**, 309–72.
VAZ, Z. and C. PEREIRA (1930). *C.R. Soc. Biol.*, Paris, **103**, 1315–17.
WALLIN, IVAN E. (1909). *Stud. zool. Lab. Univ. Neb.* No. 96.

EXPLANATION OF PLATES

PLATE VIII

Fig. 1. *Helicometra tenuifolia* n.sp. Entire animal.
Fig. 2. *Helicometra tenuifolia*. Genital end-organs, male.
Fig. 3. *Helicometra bassensis* n.sp. Entire animal.
Fig. 4. *Helicometra bassensis*. Genital end-organs, male.
Fig. 5. *Dactylostomum gracile*, n.g. et n.sp. Entire animal.
Fig. 6. *Parahemiurus australis* n.sp.

PLATE IX

Fig. 7. *Erilepturus tiegsi* n.sp.
Fig. 8. *Otodistomum pristiophori* (S. J. Johnston).
Fig. 9. *Probolitrema philippi* n.sp. Entire animal.
Fig. 10. *Probolitrema philippi*. Genital end-organs.
Fig. 11. *Probolitrema antarcticus* n.sp. Entire animal.
Fig. 12. *Probolitrema antarcticus*. Genital end-organs.
Fig. 13. *Probolitrema antarcticus*. Ova highly magnified, showing yolk granules.
Fig. 14. *Probolitrema antarcticus*. Horizontal longitudinal section, female reproductive organs.

Key to Lettering

Cr, cirrus; *Cs*, cirrus sac; *Dc*, dorsal commissure; *De*, ductus ejaculatorius; *Dh*, ductus hermaphroditicus; *Ev*, excretory vesicle; Ex_1–Ex_4, excretory branches; *Ga*, genital atrium; *Gd*, genital depression; *Gp*, genital pore; *Gpp*, genital papilla; *Int*, intestine; *Jn*, junction of male and female ducts; *Lc*, Laurer's canal; *Mp*, muscular propulsive portion; *Mt*, metraterm; *N*, nucleus; *Nu*, nucleolus; *Ot*, ootype; *Ov*, ovary; *Pc*, prostatic cells; *Pp*, pars prostatica; *Rs*, receptaculum seminis; *Sg*, shell gland; Sp_1–Sp_2, sphincters; *Ss*, sinus sac; T_1, anterior testis; T_2, posterior testis; *Ts*, testicles; *Ut*, uterus; *Vd*, vasa deferentia; *Vg*, ventral groove; *Vs*, vesicula seminalis; *Vt*, vitellaria; *Vv*, valve; *Yd*, yolk duct; *Ydm*, median yolk duct; *Y*, yolk granules; *Yr*, yolk reservoir.

(*MS. received for publication* 25. VI. 1934.—Ed.)

PARASITIC COPEPODS OF FISH FROM CEYLON

By P. KIRTISINGHE

Demonstrator in Zoology, University College, Colombo, Ceylon

(With 46 Figures in the Text)

The descriptions by Bassett-Smith (1898–9), Thompson and Scott (1903) and Wilson (1906) constitute practically all the work which has been done on the interesting group of Copepoda parasitic on fishes along the Ceylonese coast. To this short recorded list the present author has added five new species (1932–4). In a short series of papers, of which this is the first, it is proposed to describe a number of species, some of which are new forms, collected since those dates.

(1) **Pennella diodontis** Oken (1816)

Host and record. One adult and three immature females were obtained from the porcupine fish *Diodon maculatus* cast up on the beach in Colombo in November 1933. They were not restricted to any particular part but scattered over the body of the host, the head of the parasite in every instance lying between the skin and the coelomic epithelium. All the specimens are broken in two, so that the external free portion and the part of the parasite within the host are in separate pieces, but they are nevertheless complete.

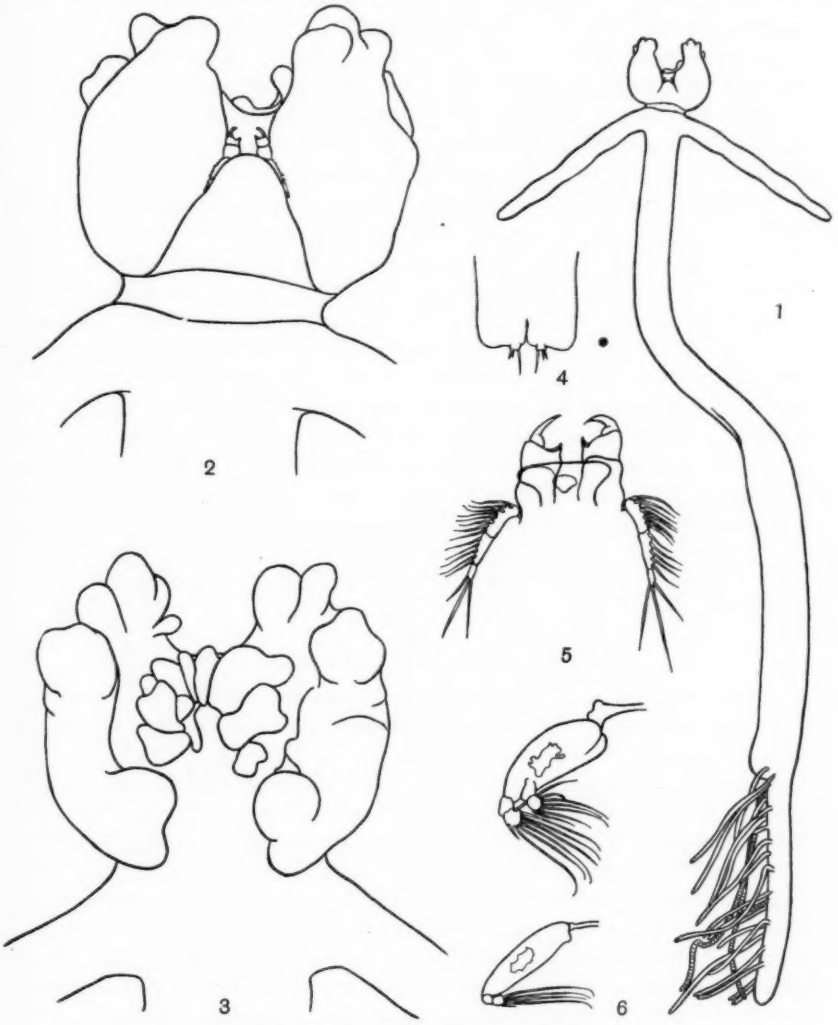
Description of adult female (Fig. 1). The head, distinctly marked off from the thorax by a groove, is wider than long, dorso-ventrally flattened and hollowed out in the anterior middle line. The papillae are arranged in two sets, a larger outer set and a smaller inner set on either side of the mouth opening (Fig. 3).

A pair of lateral thoracic horns extend diagonally outward and backward. They are more than twice as long as the head, have a nodulated appearance and taper towards their extremities. The neck is fairly long, of a more or less uniform diameter and passes imperceptibly into the stouter, cylindrical genital segment. The abdomen has a diameter of about half that of the genital segment and bears along each side of it a row of slender, unbranched processes. At its extremity the abdomen is bilobed and a short, stumpy anal lamina with three setae is present on each lobe (Fig. 4). The inner seta of each lamina is longer than the two outer setae.

The egg strings are a little longer than the abdomen.

The first antennae are slender and three-jointed with setae on their outer faces and on the tips of the distal joints. The second antennae, also three-jointed, end in powerful claws which shut down against a projection on the inner distal edge of the second joint. There is a minute spine on the base of

each claw on its inner side. The four pairs of swimming legs are placed ventrally, extending a short distance backward from about the mid-level of the bases of the horns. They consist of a pigmented conical base and two-jointed rami; the



Figs. 1–6. (For explanation see p. 344.)

two anterior pairs being biramous, the other two uniramous. The flattened edge of the distal joint of each ramus is provided with a row of setae.

Dimensions (in mm.). Length of head 2; breadth of head 2·5; neck and thorax 9; genital segment 8; abdomen 5; length of horns 5; entire length 24.

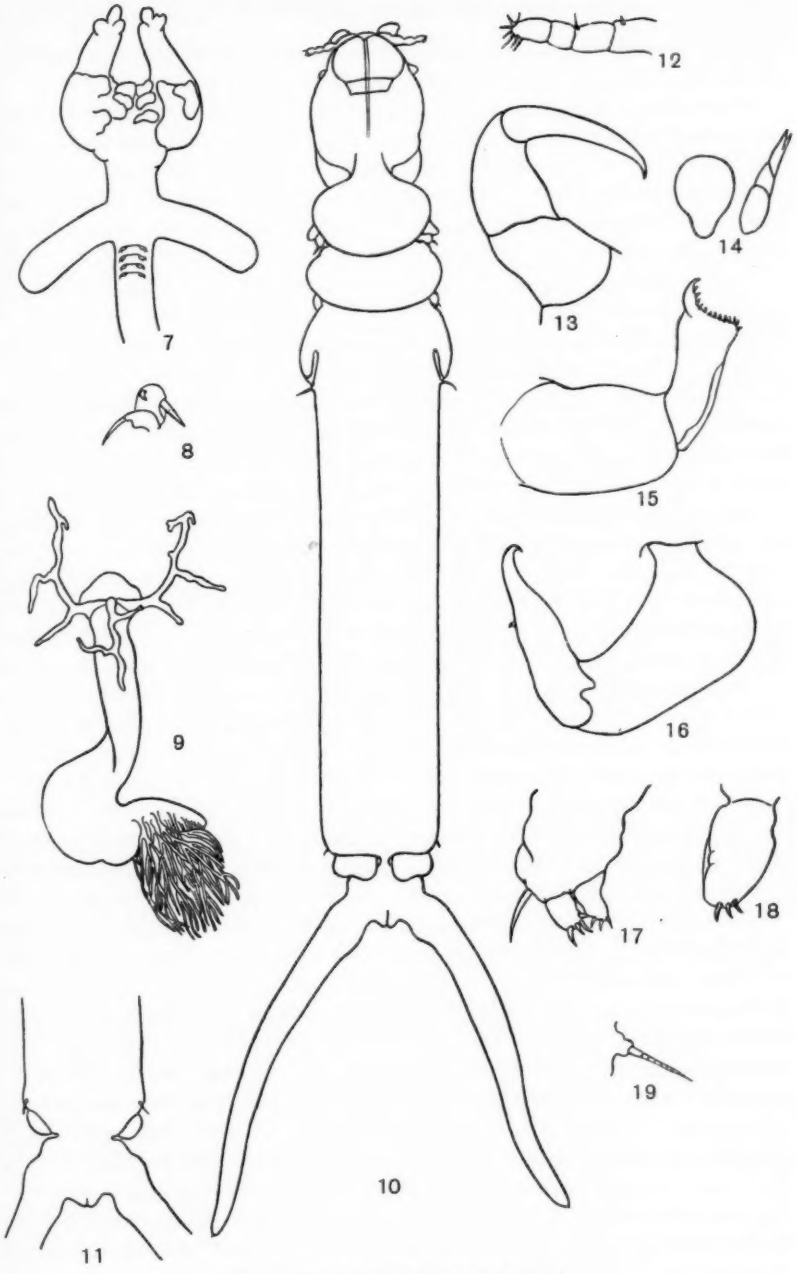
Note on the immature females. Of the three immature specimens two are without any signs of the abdominal processes, but the third shows at least the rudiments of twenty-one pairs. In all of them the hollowing out of the anterior middle region of the head is well marked—more so than in the adult. The condition of the horns seems to show that in the young they are short and stout but become longer and tapering with age. The head papillae are already formed in all of them but fortunately in one of them they were not so developed as to hide all the mouth parts and in this the maxillae are distinguishable; the first maxillae consist of a swollen base with a pair of stout pointed spines and the second maxillae are in the form of two-jointed prehensile hooks (Fig. 8).

Remarks. Wilson (1917) has made *Pennella diodontis* a synonym of *P. sagitta*. In doing this he has relied entirely on Nordmann, who considered a *P. sagitta* from *Antennarius marmoratus* to be the same as the parasite from *Diodon* sp. described by earlier writers. These two parasites have a superficial resemblance. They are apparently alike in size, in possessing only two horns and in the character of the abdominal processes. It is therefore not surprising that, where more detailed descriptions of the head, the appendages and the general proportions of the body were wanting, these two forms came to be regarded as identical. Nevertheless, all the authors [Oken (1816), Blainville (1822), Quidor (1912)] who did obtain complete specimens of the parasite from *Diodon*, described it as a distinct species from that found on *Antennarius*. Neither do the observations here recorded support the conclusion that they are identical as suggested by Nordmann (1832) and, following him, by Wilson. *Pennella diodontis* Oken is therefore retained as a separate species from *P. sagitta* Linnaeus.

(2) **Lernaeolophus sultanus** Nordmann (1864)

Host and record. A single female (Fig. 9) was found attached to the ventral body wall immediately in front of the anus of a sail-fish, *Istiophorus gladius* Broussonet, captured in February 1934.

Description. The cephalothorax is hemispherical and at right angles to the long axis of the neck. Its flattened ventral surface is provided with a few more or less symmetrically arranged swellings on either side of the median depression which marks the position of the retracted proboscis and mouth. At the level of attachment of the head and neck are given off three horns, one dorsal and two lateral, all of which are branched on much the same pattern although the laterals are a little longer than the median horn. The neck is fairly stout immediately behind the head, but narrows gradually down to the trunk or genital segment, undergoing at the same time a sinistral torsion of about 90 degrees so as to bring the curve of the trunk into the horizontal plane and to direct the ventral surface of the trunk and abdomen to the left side. The trunk is stout; the much narrower abdomen is bilobed at its extremity. The branched abdominal processes extend along either side of the abdomen and on to the posterior end of the trunk. They are arranged in two rows on each side,



Figs. 7–19. (For explanation see p. 344.)

an upper row of six processes arising dorso-laterally and a lower row of five, alternating in position with those of the upper.

The antennae are missing; they were probably broken off in clearing away the hard cyst round the head. The four pairs of swimming legs are present close together in the curve of the neck just behind the head. They consist of conical basal joint and two-jointed rami. The first two pairs are biramous, the third and fourth uniramous. The distal joint of each ramus is long and well armed with setae along the flattened edge.

The coiled egg strings are almost entirely covered by the abdominal processes.

Dimensions (in mm.). Head 3; neck 5; trunk 3; abdomen 2; entire length 13.

Remarks. The position of attachment of this specimen on the belly of its host is unusual, all previously recorded specimens having been obtained from the buccal cavity, the gill chamber or the gill arches of the host fish (Nordmann, 1864; Milne Edwards, 1840; Heller, 1865; Brian, 1912; Wilson, 1917).

The present specimen extends the known distribution of the species from the Atlantic and the Mediterranean into the Indian Ocean.

(3) **Pseudocycnus appendiculatus** Heller (1865)

Host and record. Seven females and a male were obtained from the gill filaments of the bonito *Euthynnus alletteratus* Rafinesque in May 1934.

Description of female (Fig. 10). The cephalothorax is ovate and its postero-lateral areas are prolonged into oval lobes above the basal joints of the maxillipedes. The first thoracic segment forms a narrow neck connecting the head with the second thoracic segment. The latter is as wide as the head; the third and fourth thoracic segments gradually increase in width so that the fourth is the broadest. These three segments have convex-lateral margins which are slightly produced in each segment to form a pair of dorsal laminae.

The trunk is a little narrower than the fourth segment and is about four times as long as it is wide. It has nearly straight sides and shows no trace of segmentation. Posteriorly it is provided with a pair of moderate spines placed ventro-laterally so that they are also visible in dorsal view; medially it passes behind into the narrower abdomen, and at the sides of this connection with the abdomen a pair of genital lobes are attached to the hind end of the trunk. The abdomen is about as long as it is wide. The anus is a longitudinal slit at the middle of its posterior margin and the anal laminae arise from its postero-lateral angles as rounded and tapering processes. They are not lanceolate as described by other authors (Brian, 1912; Wilson, 1922; Leigh-Sharpe, 1930). They make up nearly a third of the animal's entire length and are tipped with a minute spine. The genital lobes cover the openings of the oviducts and the bases of the egg strings which are dorsal to the abdomen in position. In ventral view these lobes are hemispherical in shape and more lateral in relation to the hind end of the trunk (Fig. 11).

The egg strings grow to be longer than the entire length of the body.

The first antennae (Fig. 12) are four-jointed. The first and second joints are stouter than the rest and each of them bears a small seta on its anterior face. The third joint is small and devoid of setae while the oval terminal joint bears seven or eight setae at its apex, the majority of which are on the posterior margin and directed backward. The second antennae are prehensile and three-jointed, the first two joints forming a stout base for the terminal elongated claw (Fig. 13). The mouth tube has the shape of an inverted pear and the first maxillae which lie to the sides of it are three-jointed; the terminal joint is bifid at the tip (Fig. 14). The second maxillae (Fig. 15) have a strong basal joint and another joint which is stouter distally than at its proximal end. Terminally this second joint has a claw-like projection on its inner side. A number of short spines are arranged along the outer side of this claw and on the broadened outer extremity of the joint. The powerful maxillipedes (Fig. 16) have a stout basal joint and a strong terminal claw bearing a short spine on the inner side near its middle.

The first pair of swimming legs is reduced to flattened laminae. The second swimming legs (Fig. 17) consist of a basal lobe with two one-jointed rami. A long spine arises from the base lateral to the outer ramus which is armed with four short spines at its apex while the inner ramus carries only three such spines. The third swimming legs are made up of an oval base with three short spines at its extremity (Fig. 18); the fourth legs project from the sides of the fused posterior body just behind the dorsal laminae of that segment as elongated spines arising from short, stumpy bases (Fig. 19).

Colour when fresh a bright red; egg strings dark brown.

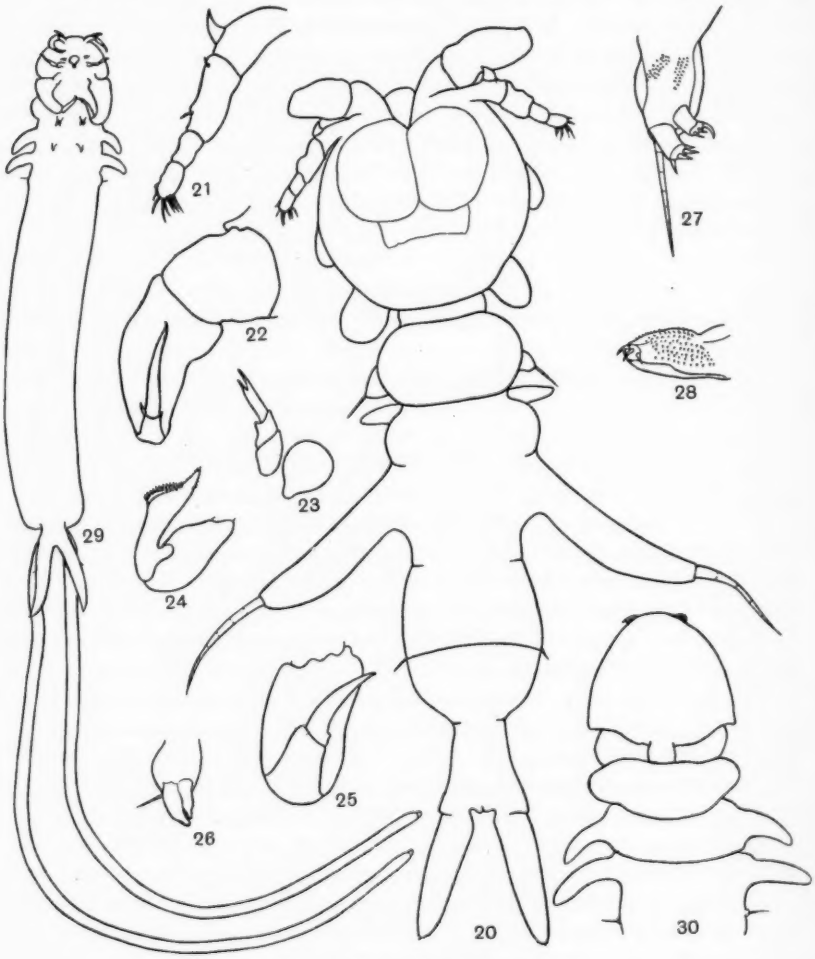
Dimensions (in mm.). Cephalothorax 1; thorax and trunk 6; abdomen and anal laminae 3; entire length 10.

Description of male. The general shape of the male in dorsal view is as shown in Fig. 20. The head is rounded. A blunt rostrum projects from it in the anterior middle line, the two pairs of antennae showing clearly on either side of it. The second maxillae are visible outside the lateral margins and the maxillipedes stand out as large lobes at the postero-lateral regions of the head.

The first thoracic segment forms a short neck which is less than half the width of the head. The second thoracic segment is wider and more than four times as long as the first. The swimming legs of this segment project outwards below its convex lateral margins. The third segment is wider than the second but not quite as long and its swimming legs project from its sides anteriorly. The fourth segment is not clearly marked off from the third but is wider. From its sides the long fourth legs extend outward and backward, forming an angle of about 60 degrees with the body axis.

The trunk, formed of the fifth and genital segments, is sub-oval in shape. The fifth segment is longer than the genital segment and its postero-lateral corners are provided with two jointed setae. The abdomen is longer than the genital segment. Anteriorly it is less than half the width of the latter but

becomes broader behind, with concave lateral margins and a sinus at the middle of its posterior margin. The anal laminae which arise from its posterior sides are longer than the abdomen. They are rounded and bluntly tapering, some-



Figs. 20–30. (For explanation see p. 344.)

what divergent and armed at the tips with a number of minute spines, one of which at the apex is larger than the others.

The first antennae (Fig. 21) are four-jointed. The first joint carries a stout spur-like spine on its anterior face; the second joint bears one or two setae and the oval terminal joint is furnished with seven or eight setae arranged as in the female. The second antennae (Fig. 22) are strongly prehensile and consist of a

short, stout, basal joint, a longer second joint and a terminal elongated claw with two spines at its base, one posterior to it, the other, slightly larger, on its inner side, both these spines arising from the distal end of the second joint. The first maxillae and mouth tube (Fig. 23) closely resemble those of the female, but the second maxillae (Fig. 24) are rather different in that their second joint, drawn out at the inner distal end to form an acute point with small spines on either side of it, does not quite possess the claw-like structure of that of the female, and the outer portion of the distal end has a swollen appearance, bearing a number of short spines arranged in several rows. The maxillipedes (Fig. 25) are composed of a very stout basal joint and a strong terminal claw.

The first swimming legs (Fig. 26) consist of a basal joint with two small, flattened, lobe-like rami, the outer of which is slightly larger than the inner. The tips of the rami are provided with very minute spines and a seta is present on the basal joint. The second swimming legs (Fig. 27) are made up of a basal joint with a long, jointed seta arising from it on the outside, and two one-jointed rami, the outer of which carries four spines at its apex while the inner, slightly smaller ramus, carries only three such spines. The basal lobe is covered ventrally with numerous short spines. The third swimming legs (Fig. 28) have the form of an oval lobe covered with small spines and carrying three larger spines at its distal end. The fourth legs are lateral cylindrical prolongations of their segment carrying a long jointed seta at the ends.

Colour in 4 per cent. formalin a yellowish white.

Dimensions (in mm.). Length of head 0·8; thorax and trunk 1·7; abdomen and anal laminae 1; total length 3·5.

(4) **Pseudocycnus armatus** Bassett-Smith (1898)

Host and record. The collection consists of over fifty females from the gills of the seer fish *Scomberemorus commersoni* Lacepede (=*Cybium commersoni*) taken in May 1934. One fish was observed to be infested with more than forty parasites. Although they attach themselves to the filaments of any of the gills they are generally more numerous on the filaments of the hyoidean hemibranch.

Description of the female (Fig. 29). The head is rounded in front, more or less truncated behind. The first thoracic segment is reduced to form a short and narrow neck connecting the head with the second thoracic segment, the basal joints of the maxillipedes being visible in dorsal view on either side of this neck (Fig. 30). The second thoracic segment is as broad as the head and has convex lateral margins. The third and fourth segments are drawn out laterally into prominent, rounded, bluntly tapering processes.

The trunk is as broad as the head and is in the form of an elongated cylinder, without any signs of segmentation. The egg strings are attached to its extremity, dorsal to the base of the abdomen. When fully mature these are more than twice as long as the body. The abdomen is less than half the width of the trunk and bifurcates behind into rounded, tapering anal laminae. These are not

distinctly separated off from the abdomen. Each lamina is provided with a short spine at its tip and another at about its middle on the outer side.

The first antennae (Fig. 31) are made up of seven joints of which the distal joint is setose. The second antennae (Fig. 32) are larger and prehensile. They are three-jointed, the terminal joint being a strongly curved claw with a dentate projection at about its middle on the inner side. The mouth tube is flask-shaped and contains the styliform mandibles (Fig. 33). The first maxillae (Fig. 34) are small and two-jointed. The second maxillae (Fig. 35) also two-jointed, are larger and their terminal joint is drawn out into a claw-like projection the concavity of which is covered with slender spines. The maxillipedes (Fig. 36) are strongly prehensile. They are three-jointed; the first joint is short, the second joint is very stout and filled with muscles attached to the terminal powerful claw which bears a dentate projection at about a third of its length from the tip on the inner side. The outer face of the second joint is provided with tufts of short, hair-like setae.

The first pair of swimming legs is indistinguishable. The second pair (Fig. 37) consists of a broad base covered with short setae giving it a ciliated appearance, and two one-jointed rami: each ramus is tipped with a short spine and two much smaller spines and a long jointed spine arises from the base of the leg lateral to the outer ramus. The third pair of swimming legs (Fig. 38) has a single ramus tipped with a short spine and two other minute spines on the inner side. The fourth pair of legs (Fig. 39) is represented by elongated spines arising from short stumpy bases.

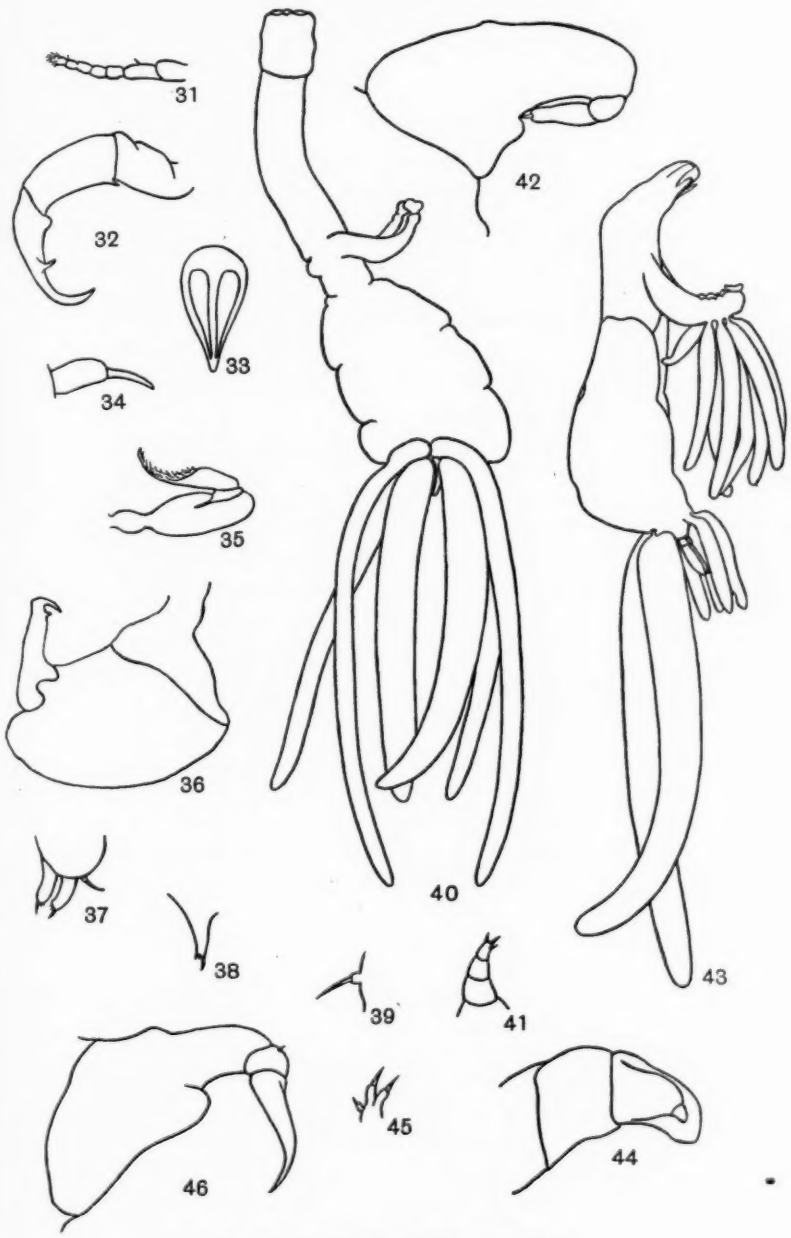
When fresh the colour of the head is whitish yellow and the trunk red. Inside this red trunk the oviducts stand out clearly on account of their brown colour. The egg strings are a muddy brown.

Dimensions (in mm.). Head 1; thorax and trunk 4·5; abdomen and anal laminae 1·5; entire length 7.

Remarks. The only previous description of this species is by Bassett-Smith (1898 *a*) who obtained it from the gills of a *Cybium guttatum* at Bombay. He called it *Helleria armata* but later (1898 *b*) altered the name of the genus to *Cybicola*.

There does not appear to be any need or justification for placing this form in a genus by itself. The differences between it and the two other species of *Pseudocycnus* known are not of generic rank. Bassett-Smith was in error when he interpreted its thoracic region to be composed of only three segments bearing three pairs of swimming appendages of which the first is biramous, the second uniramous and the third stump-like. Actually the first thoracic segment is greatly reduced as described and figured above and the pair of swimming legs of this segment has entirely disappeared. This segment with its appendages is also much reduced in *P. appendiculatus* and is almost entirely indistinguishable in *P. buccatus* Wilson (1922). The three segments of the thorax described by Bassett-Smith are, therefore, the second, third and fourth.

The presence of a pair of lateral processes on each of the third and fourth



Figs. 31–46. (For explanation see p. 344.)

thoracic segments is a characteristic of this species. That this does not call for any marked novelty in structure is obvious for if, theoretically, these processes be bent backwards so as to lie pressed against the side of the segment and are then covered over with a thin layer of chitin, they assume the appearance of the dorsal laminae of the fourth thoracic segment of *P. appendiculatus*.

(5) **Brachiella thynni** Cuvier (1830)

Host and record. Two females were obtained from the base of the pectoral fin of a yellow-finned albacore *Thunnus* (*Germo*) *macropterus* Temminck and Schlegel in July 1934.

Description of the female (Fig. 40). The cephalothorax is as long as the trunk. The head is flattened and covered with a dorsal carapace. The neck is only slightly narrower than the head, cylindrical and of the same diameter throughout. It is separated from the trunk by a distinct groove. The trunk is ovate, flattened dorso-ventrally and three segments are indicated in it by lateral indentations. The ventral surface of the trunk is marked by longitudinal furrows or pits on either side of a median ridge which latter is also marked by narrow transverse grooves. A small, median genital process is present at the end of the trunk and there are two pairs of posterior processes, one dorsal and the other ventral to the egg sacs. The ventral processes may be as long as the egg sacs or a little longer; the dorsal processes are longer than the ventral.

The first antennae (Fig. 41) are small and made up of four short joints, the terminal joint being armed with a few minute setae. The second antennae are pressed against the anterior margin of the head and are visible in front of the carapace in dorsal view. They are three-jointed with a small two-jointed exopodite. The first maxillae are tripartite, each part consisting of a swollen base tipped with a spine. The second maxillae are comparatively short and free to the tips; each of them ends in an attachment disc; the two discs joined together without forming a bulla. The maxillipedes (Fig. 42) stand out like a lower lip. They consist of a stout base followed by a short joint and then a longer, cylindrical joint directed backward along the inner side of the basal joint and tipped with a spine.

Colour of specimens white.

Dimensions (in mm.). Length of cephalothorax 5; breadth of cephalothorax 1; length of trunk 5; greatest breadth of trunk 3; length of egg sacs 6; length of dorsal processes 9.

Remarks. This species is typically a parasite of the genus *Thunnus* South. Thompson and Scott (1903), however, record it from the gills of the Indian dorab *Chirocentrus dorab*, but as they give neither description nor figures their identification remains to be verified.

(6) **Thysanote appendiculata** Steenstrup and Lütken (1861).

Host and record. Two females were obtained from the gill arches of the dark pomfret *Stromateus fiotola* Bloch in July 1934.

Description of the female (Fig. 43). The cephalothorax is short and stocky. The head is somewhat flattened but not enlarged and is without a dorsal carapace. The neck is separated from the trunk dorsally and on the sides by a faint groove. The ovate trunk is marked by two shallow grooves so as to indicate three segments in it. A pair of anal laminae is present and four short posterior processes, two on each side, arise ventrally to the egg sacs which are long and contain each about twelve longitudinal rows of eggs.

The first antennae are small, apparently three-jointed and tipped with a single seta. The second antennae (Fig. 44) carry two-jointed exopods and are turned inwards below the frontal margin. The first maxillae are bifid, each half and the palp tipped with a spine (Fig. 45). The second maxillae are stout and entirely free to their tips. They are marked by a few wrinkles on their distal half. The bulla is shallow. From the posterior side of each maxilla depend four long, unbranched processes. The maxillipedes (Fig. 46) have a stout base with a blunt projection on its inner side, a second short joint provided with a small spine on its outer face, and a terminal elongated claw.

The colour is milky white.

Dimensions (in mm.). Length of cephalothorax 2; length of trunk 3; greatest breadth of trunk 2; length of egg sacs 6.

REFERENCES

BASSETT-SMITH, P. W. (1898 *a*). Some new parasitic Copepods found on fish at Bombay. *Ann. Mag. nat. Hist.* ser. 7, **1**.

—— (1898 *b*). Some new or rare parasitic Copepods found on fish in the Indo-tropical region. *Ibid.* ser. 7, **2**.

BLAINVILLE, MARIE H. D. DE (1822). Mémoire sur les Lernées. *J. Phys. Radium*, **95**.

BRIAN, A. (1912). *Résultats de Campagnes Scientifiques de S.A.S. le Prince Albert Ier de Monaco* (1886–1910), **38**.

CUVIER, G. L. (1830). *Le Règne Animal.* Éd. 2. Paris.

HELLER, C. (1865). *Reise der Novara.* Wien.

KIRTISINGHE, P. (1932). *Pennella zeylanica* n.sp. A parasitic Copepod of *Histiophorus gladius* Day. *Parasitology*, **24**, 137.

—— (1933). Two new parasitic Copepods from Ceylon. *Ibid.* **24**, 548.

—— (1934). *Gloiopotes watsoni* n.sp. and *Lernaeenicus seeri* n.sp. Parasitic Copepods of fish from Ceylon. *Ibid.* **26**, 167.

LEIGH-SHARPE, W. H. (1930). *Résultats Scientifiques du Voyage aux Indes Orientales Néerlandaises*, **3**.

MILNE EDWARDS, H. (1840). The Lernaeans. *Histoire Naturelle des Crustacés*, **3**.

NORDMANN, A. von (1832). Erster Beitrag zur Naturgeschichte der Lernäen. *Mikr. Beitr. Naturg. wirbel. Thiere*, Pt. 2.

—— (1864). *Bull. Soc. Imp. Nat. Moscou*, **37**.

OKEN, L. (1815). (Pub. 1816.) Parasitic Copepods. *Lehrbuch der Naturgeschichte*, **2**.

QUIDOR, A. (1912). Copépods Parasites. *Deuxième Expédition Antarctique Française.*
STEENSTRUP, J. J. and LÜTKEN, C. R. (1861). *K. Danske vidensk. Selsk. Skr.* ser. 5, **5**.
THOMPSON, I. C. and SCOTT, A. (1903). *Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar*, Pt. 1.
WILSON, C. B. (1906). *Ibid.* Pt. 5.
—— (1917). North American parasitic Copepods belonging to the Lernaeidae with a revision of the entire family. *Proc. U.S. Nat. Mus.* **53**.
—— (1922). North American parasitic Copepods belonging to the family Dichelesthiidae. *Ibid.* **60**.

EXPLANATION OF FIGURES

Figs. 1–5. *Pennella diodontis* ♀. 1, entire animal; 2, dorsal view of head; 3, ventral view of head; 4, anal laminae; 5, first and second antennae.

Figs. 6–8. *Pennella diodontis* ♀. 6, second and third swimming legs; 7, ventral view of head and horns of juvenile form; 8, first and second maxillae of juvenile form.

Fig. 9. *Lernaeolophus sultanus* ♀. Entire animal, dorsal view.

Figs. 10–19. *Pseudocycnus appendiculatus* ♀. 10, entire animal, dorsal view; 11, ventral view of hind end of trunk, genital lobes and abdomen; 12, first antenna; 13, second antenna; 14, mouth cone and first maxilla; 15, second maxilla; 16, maxillipede; 17, second swimming leg; 18, third swimming leg; 19, fourth swimming leg.

Figs. 20–28. *Pseudocycnus appendiculatus* ♂. 20, entire animal, dorsal view; 21, first antenna; 22, second antenna; 23, mouth cone and first maxilla; 24, second maxilla; 25, maxillipede; 26, first swimming leg; 27, second swimming leg; 28, third swimming leg.

Figs. 29–39. *Pseudocycnus armatus* ♀. 29, entire animal in ventral view; 30, dorsal view of head and thoracic segments; 31, first antenna; 32, second antenna; 33, mouth cone and mandibles; 34, first maxilla; 35, second maxilla; 36, maxillipede; 37, second swimming leg; 38, third swimming leg; 39, fourth swimming leg.

Figs. 40–42. *Brachiella thynni* ♀. 40, entire animal; 41, first antenna; 42, maxillipede.

Figs. 43–46. *Thysanote appendiculata* ♀. 43, entire animal; 44, second antenna; 45, first maxilla; 46, maxillipede.

(*MS. received for publication* 26. XI. 1934—Ed.)

ERRATA

Parasitology, **26**, 169–72

For first maxilla *read* maxillary hook.
For second maxilla *read* first maxilla.
For first maxillipede *read* second maxilla.
For second maxillipede *read* maxillipede.

ON THE PERSISTENCE OF EOSINOPHILIA, AND ON IMMUNE REACTIONS IN HUMAN TRICHINOSIS, SEVERAL YEARS AFTER RECOVERY

By HANS THEILER, DONALD L. AUGUSTINE and WESLEY W. SPINK

Department of Comparative Pathology, Medical School and School of Public Health, Harvard University

The significance of the discovery of the eosinophilia in trichinosis (Brown, 1897, 1898, 1899) was immediately recognised by clinicians such as Thayer (1897), Cabot (1897) and Osler (1899). Brown's findings were soon confirmed, and the demonstration of an eosinophilia has remained a valuable aid in diagnosing trichinosis. In the course of time attempts were made to apply serological methods to parasitic infections and in certain instances met with considerable success, and Bachman (1928, 1928 *a*) showed that specific precipitin and skin tests could be obtained in experimental trichinous infections in laboratory animals. Stoll (1929) and Hunter (1931) applied the precipitin test in human cases, and Augustine and Theiler (1932) confirmed Bachman's findings in laboratory animals and extended their observations to an investigation of the precipitin and skin tests as aids to the diagnosis of trichinosis in human and porcine subjects. The latter authors found the tests to be highly specific when correctly applied. McCoy, Miller and Friedlander (1933) found that the intradermal test possessed a high degree of specificity, but felt that under some circumstances a negative result would be of relatively greater value in ruling out the disease. Maternowska (1933) concludes that the intradermal reaction is specific and diagnostic in laboratory animals, man and swine. Kilduffe (1933), apparently unaware of the work of Augustine and Theiler, and of McCoy, Miller and Friedlander, and using the technique and criteria of Bachman (1928), obtained indifferent results with the intradermal test in man and concluded that it presents no practical advantages over the demonstration of an eosinophilia. Friedlander (1934) draws attention to the fact that Kilduffe attempted to make use of the delayed reaction and a 1 : 100 dilution of the antigen and not of the immediate reaction with the greatly diluted antigen, which gave such excellent results in the hands of other workers. More recently Trawinski and Maternowska (1934) have published a study of the occurrence of precipitins in experimental infections in rabbits.

The cases studied by Augustine and Theiler (1932) came under observation at various intervals of time up to 6½ months after infection. The present paper contains observations on known trichinous patients 4–9 years after their admission to the Boston City Hospital.

Observations

Letters were sent to about fifty persons, previously patients of the Boston City Hospital, against whose names a discharge diagnosis of trichinosis had been entered in the records. Of these, eight reported on the appointed days and seven agreed to undergo the tests. Blood smears for differential counts were made with drops of blood obtained by puncture of the ear lobe, about 5 c.c. of blood for serum for the precipitin tests drawn from the median basilic vein and the skin test performed. As in the earlier work, the skin test was made by injecting 0·1 c.c. of the alkaline phenolised saline extract of dried powdered larvae of *Trichinella spiralis* into the skin of the forearm. In the present series of tests the antigen was diluted to 1 : 10,000 (in terms of dry powder). The control injection, consisting of an equal amount of the extracting fluid (Coca's solution), was injected into the corresponding site on the other forearm. Differential counts were made. The precipitin tests were set up the following day, 0·2 c.c. of the serum was overlaid with the same amount of a 1 : 100 dilution of the trichinella extract. A reading was made after 1 hour, the tubes shaken up, incubated for 1 hour in the water-bath, stored overnight in the cold room and again read the following morning. The data are summarised in tabular form (Table I).

While there can be no doubt of the correctness of the diagnosis of trichinosis in the first five cases, the evidence in Nos. 6 and 7 is less clear. We were not aware that case No. 6 was wrongly entered in the hospital records as a case of trichinosis until after the tests were performed. In the course of the interview, this patient informed us that she was in the habit of eating a type of raw pork sausage. This patient is an Italian woman, and at the time of hospitalisation was a recent immigrant, but had no doubt become infected both before her arrival in this country and since her hospitalisation in 1927. In the other case, No. 7, the evidence appears somewhat stronger, despite the fact that no eosinophilia could be demonstrated at four different examinations. Brown and Thayer in their original communications reported that considerable fluctuation in the eosinophile count may take place during the course of a trichinous infection. These observations were confirmed by Schleip (1904) and by Stäubli (1909). Stäubli further affirms that there may be a considerable reduction in the number of eosinophiles in very severe cases of trichinosis or in cases of trichinosis complicated by an acute bacterial infection. Case No. 7 was thus probably a trichinous infection complicated by acute tonsilitis and acute rheumatic fever.

Table I

Case	Name	Age and sex	Hospital records: Admission date	Hospital records: History and symptoms	Hospital records: Eosinophilia %	Present findings: Date	Present findings: Skin	Present findings: Precipitation	Present findings: Eosinophilia %
1	M.C.	13 F.	Dec. 4, 1922	Pork eaten. Typical. Two sisters and mother affected	20	Jan. 15, 1932	Negative	Negative	Nil
2	R.W.	33 M.	Dec. 2, 1924	Myalgia. Fever. Biopsy positive. Wassermann positive	29	Feb. 4, 1932	Negative	Negative	Not made
3	W.S.	44 M.	Jan. 27, 1925	Myalgia. Fever. Conjunctiva injected. Rales. Knee jerk absent. Persistent hiccough. Biopsy positive	33	Jan. 22, 1932	Positive	Positive*	5
4	P.M.	30 M.	June 9, 1926	Pork eaten. Typical	43	Jan. 22, 1932	Positive	Positive*	5
5	F.G.	47 M.	Apr. 18, 1927	Born Italy. Abdominal pain, diarrhoea, fever. No muscular symptoms. Habitually ate raw beef and pork	16	Jan. 22, 1932	Positive	Positive*	2
6†	J.T.	35 F.	June 9, 1927	Two months pregnant. Vomitus contained Ascarid. Faecal examination—*Ascaris lumbricoides, Trichuris trichiura*	0	Jan. 22, 1932	Positive	Positive*	2
7	K.M.	15 F.	Nov. 25, 1927	Roast pork few days before onset. Muscular tenderness, weakness, fever. Developed follicular tonsilitis in hospital. Discharge diagnoses: acute tonsilitis, acute rheumatic fever, trichinosis (?)	0‡	Jan. 22, 1932	Positive	Doubtful§	7

* Positive by flocculation, no definite ring.

† Patient states she habitually eats a type of raw pork sausage.

‡ Four examinations made.

§ Serum cloudy.

Discussion

Numerous authors since Brown (1897, 1898) have written on the subject of the eosinophilia encountered in trichinosis. Schleip (1904) reported that the eosinophilia appeared on the fourteenth or fifteenth day of illness, and Brown, Thayer, Schleip, and Stäubli (1909) all affirm that considerable fluctuations in the relative and absolute number of eosinophiles may be encountered in the course of an infection. A considerable decrease and often a complete disappearance of eosinophiles may occur in very severe cases or in cases of trichinosis complicated by febrile conditions due to septic or other causes (Stäubli). Spink (1934) reports a case of trichinosis complicated by a pyogenic infection in which the eosinophilia did not appear till healing of the pyogenic lesions occurred. The diagnosis in this case rested on the demonstration of the eosinophilia, verified by positive skin and precipitin reactions and confirmed finally by a positive biopsy. Spink's work in experimental animals further shows that the eosinophilia due to infection with *Trichinella spiralis* may be decreased or suppressed by a superimposed bacterial or protozoan infection or may be increased by such influences as dry heat or injections of foreign protein (killed bacteria). We have found no references on the effect of pregnancy on the eosinophilia of trichinous infections in the human subject. An incomplete search of the literature has yielded little information bearing on the duration of the eosinophilia after recovery from trichinosis. In two of Stäubli's cases the count at the end of 3 months was 8 and 7 per cent. Stäubli further reports 24 per cent. eosinophiles in a guinea-pig after 6½ months and 8 per cent. in another animal 3½ years after infection. In a report to Stäubli, Giesböck records an eosinophile count of 7 per cent. in one patient at the end of 1 year and 7·1 per cent. in another patient at the end of 6 months.

Brown (1898 *a*) saw one of his first three cases 6 months after discharge from the hospital. At that time, January 22nd, 1898, the eosinophile count was 34·7 per cent., and on July 10th, 1898, it had fallen to 3 per cent. Weindrach (1929) presents blood counts on three cases of trichinosis, with observations extending over periods up to 3 years and 7 months after infection. The counts show a gradual decrease from 52, 52 and 47 per cent. to 2, 8 and 8 per cent. respectively.

In the present series the eosinophile count ranged from 2 to 7 per cent. 4–7 years after hospitalisation (see Table I). No observation was made in one case of 7 years' standing and no eosinophiles were seen in another case 9 years after infection. In general, it would appear that the eosinophilia tends to persist as long as the antibodies, but the observations made are too few in number to warrant any conclusion being drawn.

In contrast to the earlier cases studied by Augustine and Theiler, none of the sera of this series yielded positive readings to the "ring" test, but readings were obtained by shaking up the tubes, incubating for 1 hour in the water-bath at 37° C., storing overnight in the cold room and noting the occurrence

or absence of flocculation the following morning. No flocculation was encountered in any of the control tubes, in which Coca's solution had been added to the serum. Normal sera gave no flocculation. The demonstration of the presence of precipitins by flocculation corresponded accurately with the skin reactions obtained and negative results were recorded only in the two cases (Nos. 1 and 2) which failed to give a skin reaction. Other work in progress at the present time appears to indicate that there is never any great abundance of free antibodies in the circulating blood of trichinous subjects, and the results obtained in this study clearly indicate that they are less readily demonstrable in cases of long standing than in the earlier series reported by Augustine and Theiler. There appears, however, to be a more marked tendency for the persistence of the "fixed" antibodies, for, as mentioned above, the skin reactions obtained, though not severe, were as characteristic and as easily read as those in the earlier series. This more marked persistence of the "fixed" as opposed to the "free" antibodies is in accord with similar tendencies observed in the immunological response in certain bacterial infections, and this result was not unexpected.

Trawinski and Maternowska (1934) studied the occurrence of precipitins in three series of rabbits which had been fed varying numbers of larvae of *Trichinella spiralis*. These authors use a tedious and cumbersome method to obtain the larvae from the muscles of mastication of heavily infected rabbits, and it is difficult to conceive that sufficient material could have been obtained by this means to prepare enough of the saline extract which served as antigen in tests on sera from eighty-three experimental animals, an equal number of controls and a number of other tests. Control tests were made with an ascaris antigen and an echinococcus antigen. Precipitins were not demonstrated in the sera from four rabbits 19, 20, 24 and 34 days after infection. With one exception sera from uninfected rabbits gave no reactions. This animal was one in which the authors failed to demonstrate an infection despite the feeding of 3000 larvae 18 days previous to the post-mortem examinations. The authors failed to produce infections by feeding larvae in ten rabbits. Such an occurrence is unparalleled in our experience, and to the best of our knowledge has never been recorded before. An analysis of the results obtained by these investigators shows that the presence of *Trichinella spiralis* was correctly indicated by precipitin tests in 88·6 per cent. of the animals. A more detailed discussion of the work of these authors will be published elsewhere.

Augustine and Theiler (1932) found that there was no marked difference in the intensity of the reactions elicited by the skin test in subjects early in the infection or in those obtained 6 months after infection. Reactions were obtained in the present series of patients from about 4¼ to 7 years after hospitalisation for trichinosis. These include the reaction obtained in a patient admitted to the hospital in 1927 (Case No. 6) and erroneously included in the present series. This, however, despite the absence of an eosinophilia both in 1927 and at the present time, does not necessarily mean that the patient has

not been subject to trichinous infection. In view of the findings of Williams (1901), who demonstrated larvae of *Trichinella spiralis* in 5·3 per cent. of 505 unselected autopsies, of Queen (1931), who found the larvae in 17·5 per cent. of 344 consecutive autopsies in Rochester, New York, and in 27·6 per cent. of 58 in Boston, and of Riley and Scheifley (1934), who found larvae in 17·9 per cent. in a series of 117 cadavers, there being 20 per cent. in one series of fifty cadavers, and also in view of the patient's acknowledged predilection for raw pork sausage, it seems probable that this woman is subject to continual reinfection and hence an immunological response is to be expected. The primary infection, possibly a very light one, may have taken place early in life and subsequent reinfections have occurred regularly enough to maintain a high degree of resistance, if not an absolute immunity. It would appear that one infection results in considerable protection against reinfection (Ducas, 1921; McCoy, 1931). How long such partial immunity or increased resistance to reinfection may persist is not known, and the effect of continued reinfection over a long period of time also calls for further investigation. A study of the haematological picture under such conditions should also be undertaken. We, are, however, of the opinion, despite the lack of clinical evidence, the absence of an eosinophilia, and the omission of tests against other proteins, that the positive skin and precipitin reactions and the acknowledged dietary habits constitute good presumptive evidence that this woman is at the present time harbouring an infection, or that she has been reinfected sufficiently recently for the detection of the residuum of the immunological response to the last previous infection. Two undoubted cases (Nos. 1 and 2) of trichinous infection failed to respond to either of the tests approximately 9 and 7 years respectively after infection.

The work of McCoy, Miller and Friedlander (1933) covers observations on skin tests on eighty-eight known trichinous individuals, 104 controls from Rochester, New York, forty-seven from San Francisco, California, and ninety-two helminth infected persons from southern Louisiana. The results recorded by these authors show general agreement with those obtained by Augustine and Theiler, but the former using 1 : 10,000 dilutions of the antigen failed to get reactions in a number of known trichinous individuals and encountered a considerable number of "pseudo-positives" in their control and helminth-infected persons. The results obtained in tests made with an antigen dilution of 1 : 500 while yielding a higher percentage of positives in the known trichinous series also yield an unduly high number of reactions in the non-trichinous series.

Possibly some of the discrepancies may have to be attributed to the use of a buffered saline solution as an extracting fluid in place of Coca's solution. The fact that dilutions are made in terms of the weight of the powder before ether extraction probably introduces an avoidable source of error, as it is more than likely that different batches of material will contain differing amounts of ether-soluble substances. The question of the diagnostic value of

the intradermal test is raised, and the authors lose sight of the fact that no biologic test is infallible and any test employed should be considered in connection with all the other evidence obtainable and where possible confirmed or refuted by the application of other tests that may be available. At the present time no method has been elaborated for the standardisation of trichinella preparations in terms of the antigenic fraction of the material which may be obtained from the larvae, and it is thus to be expected that widely differing results will be obtained by different workers, especially when different extracting fluids are used in preparing the antigen. Though the results have been gratifyingly consistent in our hands, it is not known to what extent different batches of antigen may differ from each other, even when the same technique has been rigidly and consistently employed. In the work of McCoy, Miller and Friedlander under consideration, precipitin tests should, wherever possible, have been made on the sera of trichinous individuals who failed to react, as also on the sera of non-trichinous individuals who gave pseudo-positive skin reactions. In addition, these same groups of individuals should have been tested with an antigen prepared by the identical technique which gave such excellent results in the hands of Augustine and Theiler, and also Bachman. Failing this, the only conclusion one is entitled to arrive at is that different results were obtained with their technique.

A high incidence of unsuspected and undiagnosed trichinosis may occur in communities in which clinical cases occur (Williams, Queen, Riley and Scheifley). It was for this reason that no extensive series of tests on so-called normal persons were made by Augustine and Theiler, as it was felt no useful purpose could be served, as positive findings in the absence of necropsy or other corroborative evidence would be meaningless and contribute nothing to our knowledge of the specificity or validity of the tests concerned. It is, however, to be expected that in a series of tests, conducted in such a community, a considerable number of positive reactions would be obtained. In the work now reported it is seen that cases of long standing (in our series cases over 7 years) may fail to give any demonstrable immunological response. McCoy, Miller and Friedlander report fewer reactions in cases of $3\frac{1}{2}$–$7\frac{1}{2}$ years' standing. The number of individuals in an infected community giving positive reactions would thus be less than the number demonstrably infected at autopsy. In the present series, except for No. 6, are included only cases which required hospitalisation. These persons (except No. 6) as a result of their experience either no longer consume pork or eat it only when it has been well cooked. It thus seems probable that these individuals have not been subject to reinfection, and that the immunological picture observed is that resulting in response to the primary severe infections. To what extent this picture may be modified in light infections subject to periodic reinfections, we have no means of determining.

In discussing the results obtained in southern Louisiana, McCoy, Miller and Friedlander attribute the high number of reactions which they were able

to obtain to the presence of *Trichuris trichiura*, even though admitting that about the same degree of correlation could be obtained with Ascaris infection as with Trichuris. This conclusion appears to have been influenced by the fact that *Trichuris trichiura* is more closely related to *Trichinella spiralis*, than are any of the other intestinal parasites of man and is hence more likely to give rise to a "group" reaction. Undue weight appears to be attached to the positive reaction obtained in the trichuris-infected Filipino, and the authors fail to state how long this individual had been resident in the United States, or what his present dietary habits are. There appears to be no marked difference in the results in the trichuris-infected and trichuris-free groups. The assumption, however, is made that the trichuris-free individuals, who were sensitive to the trichinella antigen, had been infected in the past and were consequently still sensitive. On this basis 108 of the 124 persons tested either were harbouring or had harboured Trichuris, and it is not unreasonable to suppose that the remaining sixteen had also been infected at some time. Taking the group as a whole, 10·5 per cent. reacted to the 1 : 10,000 and 40·3 per cent. to the 1 : 500 dilution of the antigen. That is, more than half of the group (51 per cent.) gave positive reactions. There can be no doubt that most of these reactions should be considered false positives, but it is certain that much undiagnosed trichinosis does occur and that much of it is subclinical in type is evident from the findings of Williams, of Queen, and of Riley and Scheifley. No evidence of "group" reactions was obtained by Bachman and his collaborators (1934) who obtained no non-specific precipitin reactions to *Trichinella spiralis* antigen in a group of twenty-seven individuals harbouring *Trichuris trichiura*. So, despite the reputed absence of cases of trichinosis in Louisiana, it must be granted that some of the sensitisation encountered may have been due to trichinosis. Augustine and Theiler reported that the non-specific reactions obtained departed more and more from the typical picture as increasing dilutions of the antigen were used in making the tests. In the specific reactions the response, though less marked and less severe at the higher dilutions, was nevertheless characteristic. The only difference observed was one of degree; the nature of the response, however, remained constant. McCoy, Miller and Friedlander give no information on this point, and the inference to be made is that no difference in character between specific and non-specific reactions was observed by these authors. Further, as already pointed out above, a number of the reactions obtained are no doubt attributable to the fact that the different extracting fluid used rendered soluble some antigenic fraction of the trichinella material not present in the testing solution used by Augustine and Theiler.

As pointed out by Friedlander (1934), the intradermal test has proved a valuable aid in the diagnosis of trichinosis. This has been especially true in light infections, and the intradermal test in connection with the precipitin test has frequently made possible a positive diagnosis which could not otherwise have been made. Further evidence of the successful application of both

precipitin and skin reactions is furnished by Spink and Augustine (1934), who present thirty-five cases in only eleven of which trichinosis was diagnosed on admission to the hospital. In thirty-three out of thirty-four of these patients, positive skin tests were obtained. One was moribund and yielded a negative skin reaction but was positive to the precipitin test. In eight cases no skin reaction was obtained on admission, but gave positive tests later. All of the precipitin tests performed, nineteen in number, yielded positive results, and a few of these patients retested a year later still gave positive precipitin tests. The authors emphasise the great value of the tests, particularly in those cases which yield negative results at the first test and later become positive. We wish further to emphasise that in cases of suspected sporadic cases, all the evidence should be taken into account, without attaching too much weight on any one feature, symptom or test. Due weight should be given to the history, the symptoms, objective findings, skin reaction and precipitin test, and it must be borne in mind that a negative biopsy or a failure to find larvae in the blood or spinal fluid does not rule out a diagnosis of trichinosis.

SUMMARY AND CONCLUSIONS

Following a brief historical introduction, the results of differential counts, precipitin tests and skin reactions on seven cases of trichinosis 4–9 years after recovery are presented. Eosinophile counts ranging from 2 to 7 per cent. were encountered, but the number of cases studied does not warrant any conclusion being drawn. Positive precipitin tests and intradermal reactions were encountered in cases up to 7 years after infection. The findings are discussed, together with a consideration of the pertinent literature, and the importance of the skin reaction and precipitin tests as aids to diagnosis are emphasised in conjunction with due consideration of the history and symptomatology. It is affirmed that the tests have proved of value in establishing a diagnosis in mild cases.

It is a pleasure to acknowledge our indebtedness to Dr George R. Minot, who made the records of the hospital available to us, and to Dr W. D. Sutliff, whose continued interest and ready co-operation greatly facilitated our work.

REFERENCES

AUGUSTINE, D. L. and THEILER, HANS (1932). Precipitin and skin tests as aids in diagnosing trichinosis. *Parasitology*, **24**, 60–86.

BACHMAN, G. W. (1928). A precipitin test in experimental trichiniasis. *J. prev. Med.*, Baltimore, **2**, 35.

—— (1928 *a*). An intradermal test in experimental trichiniasis. *Ibid.* **2**, 513.

BACHMAN, G. W., MOLINA, R. RODRIGUEZ and GONZALEZ, J. O. (1934). Anomalous and non-specific reactions with *Trichinella spiralis* antigen in relation to other disease conditions. *Amer. J. Hyg.* **20**, 415–23.

BROWN, T. R. (1897). Studies on trichinosis. *Johns Hopk. Hosp. Bull.* **8**, 79–81.

—— (1898). Studies on trichinosis, with especial reference to the increase of the eosinophile cells in the blood and muscle, the origin of these cells and their diagnostic importance. *J. exp. Med.* **3**, 315–48.

—— (1898 *a*). A note on the duration of eosinophilia in trichinosis. *Boston med. surg. J.* **139**, 218.

—— (1899). The diagnosis of trichinosis by means of the great increase of the eosinophiles in the blood; report of fourth case. *Med. News*, N.Y., **74**, 12–14.

CABOT, R. C. (1897). The diagnosis of trichinosis. *Boston med. surg. J.* **137**, 676.

DUCAS, R. (1921). *L'immunité dans la trichinose.* Thèse. Paris: Jouve et Cie.

FRIEDLANDER, R. D. (1934). The present status of the diagnostic intradermal test for human trichiniasis. *Amer. J. med. Sci.* **188**, 121.

HUNTER, S. W. (1931). Human *Trichinella spiralis* infections and the Bachman precipitin test. *Amer. J. Hyg.* **13**, 311.

KILDUFFE, R. A. (1933). Bachman intradermal reaction in human trichinosis. *Amer. J. med. Sci.* **186**, 802–8.

MATERNOWSKA, IRENA (1933). Intradermale Hautreaktion bei Trichinose. *Zbl. Bakt.* Abt. 1, Orig. **129**, 284–301.

MCCOY, O. R. (1931). Immunity of rats to reinfection with *Trichinella spiralis*. *Amer. J. Hyg.* **14**, 484.

MCCOY, O. R., MILLER, J. J. and FRIEDLANDER, R. D. (1933). The use of an intradermal test in the diagnosis of trichiniasis. *J. Immunol.* **24**, 1–23.

OSLER, W. (1899). The clinical features of sporadic trichinosis. *Amer. J. med. Sci.* **117**, 251–65.

QUEEN, F. B. (1931). The prevalence of human infection with *Trichinella spiralis*. *J. Parasit.* **18**, 128.

RILEY, W. A. and SCHEIFLEY, C. H. (1934). Trichinosis of man a common infection. *J. Amer. med. Ass.* **102**, 1217–18.

SCHLEIP, K. (1904). Die Homberger Trichinosisepidemie und die für die Trichinosis pathognomische Eosinophilie. *Dtsch. Arch. klin. Med.* **80**, 1–38.

SPINK, W. W. (1934). The effects of vaccines, bacterial and parasitic infections upon eosinophilia in trichinous animals. *Arch. intern. Med.* **54**, 805–17.

SPINK, W. W. and AUGUSTINE, D. L. (1934). The diagnosis of trichinosis: with especial reference to skin and precipitin test. *J. Amer. med. Ass.* (in press).

STÄUBLI, C. (1909). *Trichinosis.* Wiesbaden: Bergmann.

STOLL, H. F. (1929). Trichinosis. *J. Amer. med. Ass.* **92**, 791.

THAYER, W. S. (1897). On the increase of the eosinophile cells in the circulating blood in trichinosis. *Lancet*, ii, 787–8.

TRAWINSKI, A. and MATERNOWSKA, I. (1934). Über Präcipitationsreaktion bei Trichinose. *Zbl. Bakt.* Abt. 1, Orig. **131**, 10–18.

WEINDRACH, G. (1929). Das Blutbild und die Eosinophilie bei Trichinose. *Folia haemat.*, Lpz., **38**, 380–4.

WILLIAMS, H. U. (1901). The frequency of trichinosis in the United States. *J. med. Res.* **6**, 64–83.

(*MS. received for publication* 28. XII. 1934.—Ed.)

THE MECHANISM OF FEEDING IN TICKS

By S. K. SEN, M.Sc.

Entomologist, Imperial Institute of Veterinary Research, Muktesar, U.P., India

(*From the Molteno Institute, University of Cambridge*)

(With 15 Figures in the Text)

I. Introduction

The mechanism of feeding in ticks has been fully described in numerous text-books. Briefly, it is believed to be as follows: After the tick has fixed itself on the host's body by means of the denticles on the ventral surface of the hypostome, the chelicerae, or the so-called mandibles, are brought into action, and the "protrusion and retraction of the shaft, together with the extension of the teeth on the digits, results in a saw-like movement which tears a hole in the skin" (Patton and Cragg, 1913). More recently, Sharif (1928) has suggested the possibility that the actual incisions in the skin of the host are, as a rule, made by the cheliceral digits and that through the incisions thus made, the proboscis (*i.e.* the chelicerae and the hypostome collectively) is pushed into the deeper tissues.

It is noteworthy that the actual mouth-opening in ticks has never been observed, so that the views advanced by the different workers in regard to the feeding mechanism in this group of arthropods must be regarded as being of an entirely inferential character. Patton and Cragg, however, have included in their well-known text-book a figure wherein the food canal is represented as being formed by the apposition of the chelicerae and the hypostome (see their Fig. 1, in Plate LXXXV, representing longitudinal section through capitulum of *Margaropus annulatus*). On the other hand, Nuttall, Cooper and Robinson (1908), as a result of intensive studies upon the anatomy of *Haemaphysalis punctata*, came to the conclusion that "the mouth-opening is not visible on external examination, being concealed between the median hypostome ventrally, the palpi laterally and the paired chelicerae dorsally". As will be seen from what has to be stated later, this statement is entirely correct, and the use of the term "external" reflects the great caution with which these authors consigned their observations to print. A similar statement has also been made by Robinson and Davidson (1913–14), according to whom "the opening of the mouth [in *Argas persicus*] lies concealed between the basal portions of the hypostome and the chelicerae and is hidden from lateral view by the bases of the palps".

The object of the present paper is to show that the feeding mechanism in ticks is different from what has hitherto been believed to be the case: that it is neither the chelicerae nor the hypostome that functions in the act of sucking blood, but that the sucking apparatus is a stylet which is a tubular continuation of the pharynx and which, to all appearance, represents the homologue of the hypopharynx of other blood-sucking arthropods. As will be described later, the stylet is extremely refractile, and this would appear to explain why it has hitherto escaped observation.

The whole of this work was carried out by the writer at the laboratories of the Molteno Institute for Research in Parasitology at Cambridge, during a period of study leave granted to him by the Government of India, on the recommendation of Mr F. Ware, Director, Imperial Institute of Veterinary Research, Muktesar, India, to whom the writer takes this opportunity to acknowledge his grateful thanks. He is also deeply grateful to Prof. George H. F. Nuttall, F.R.S. and Prof. D. Keilin, F.R.S., Director of the Molteno Institute, for their unfailing courtesy and constant encouragement during the progress of the investigation. It has been for the writer a rare privilege to be able to work in close association with these eminent authorities. Mr Cecil Warburton generously placed at the writer's disposal his great knowledge of the systematics of ticks, and for this the writer is much indebted to him.

Except where otherwise mentioned, the observations embodied in this paper are to be understood as relating to *Ornithodorus papillipes* Birula (*O. crossi* Brumpt). In interpreting certain of the structures bearing upon the general anatomy of this species, invaluable aid has been derived from Robinson and Davidson's (1913) paper on the anatomy of *Argas persicus*.

The present paper is designed to stimulate further work on the structure and mechanism of the mouth-parts of Ixodoidea. The illustrations are designed to give a faithful picture of the structures I have seen in dissections and drawn with the aid of a camera lucida, without any attempt at reconstruction or schematisation, which, it seems almost needless to say, is fraught with risks.

II. Material and methods

Living specimens of *O. papillipes* were brought by me from India. The consignment included both nymphs and adults and was obtained from the Punjab, through the courtesy of the Principal of the Veterinary College, Lahore. Prior to the date of embarkation, the ticks were given a feed of blood on rabbits, and during the progress of the investigation at Cambridge they were fed on fowls.

For the general examination of the mouth-parts, the ticks were killed by a momentary immersion in boiling water, as recommended by Robinson and Davidson, and they were then placed in a shallow dish of water and decapitated by means of a flat sharp-edged needle. After washing with two or three changes of water so as to remove all traces of blood and adherent debris, the mouth-parts were separated in normal salt solution by means of a pair of the

finest available needles that had been further sharpened. In removing particles of debris obstinately adhering to a delicate piece of tissue, it has been found to be a good plan repeatedly to flush the part with water by means of a pipette.

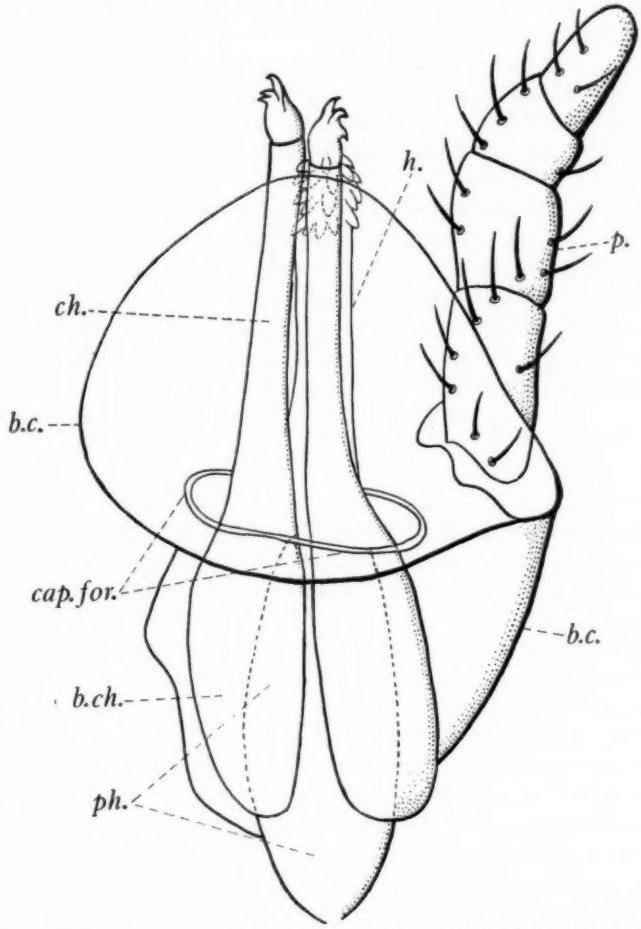


Fig. 1. *O. papillipes*. The capitulum from the dorsal aspect. One of the palpi not shown. The dorsal side of the basis capituli is dissected out and deflected to expose the basal portion of the capitulum. ×240. (*b.ch.* base of chelicera; *cap. for.* capitular foramen; other lettering as in Figs. 2–11.)

The complete isolation of the sucking apparatus in ticks is best accomplished in freshly killed specimens, although for the study of the general topography of the capitulum, dry and even alcohol specimens answer the purpose equally well, but in such cases it is advisable to keep the specimens soaked for 3–5 days in a 10 per cent. solution of caustic potash which should

be used cold. After dehydration, the parts are best orientated in a drop of cedar oil on the mounting slide.

For serial sections of the mouth-parts, the method recommended by Cox (cited by Eltringham, 1930) was found satisfactory. The parts, previously lightly stained with a trace of watery solution of eosin, were embedded in the usual way in the middle of a block of paraffin and the latter was gradually thinned out by scraping with a sharp knife, until the outline of the embedded object could be made out through the overlying layer of paraffin. The block was then carefully trimmed with due regard to the plane in which it was to be cut on the microtome.

For the study of the softer parts, such as the pharynx, a fixative was used and this was usually Bouin's fluid, but in a few instances Duboscq-Brazil solution and the so-called picro-chlor-acetic fixative were also employed. Only two stains were used: Delafield's haematoxylin and water-soluble eosin, but in a few cases the xylol-picric acid method of staining chitin was also used. The sections were cut on a Cambridge Rocking microtome and a thickness of 10μ was generally found suitable.

III. Some *a priori* considerations concerning the feeding mechanism in ticks

The normally visible appendages in the capitulum of the tick, namely, the chelicerae and the hypostome, present certain morphological features which do not appear to have received their due share of attention in relation to the feeding mechanism. In the first place, the hypostome is distinctly shorter than the paired chelicerae (Fig. 1) and it would appear difficult to explain how such conspicuously dissimilar structures could combine to form an efficient sucking tube. The possibility of their functioning as a sucking tube is further discounted by the fact that, while the hypostome is denticulate on its ventral surface, the shafts of the chelicerae are provided with chelate digits on their distal extremities, so that during the process of fixing themselves in the skin of the host, they are actually liable to diverge. As pointed out by Robinson and Davidson, the cheliceral shafts are capable of sliding only backward and forward in their sheaths, being operated in this direction by stout muscles, the tendons of which are furnished with guiding canals situated in the wall of the shafts. The rotation of the shafts in a horizontal plane is prevented by their basal portions being maintained in close apposition. With their recurved digits, and under the action of the muscles moving in an antero-posterior plane, the shafts would therefore become progressively embedded in the host's skin and ultimately completely immobilised, thereby making it difficult, as is known to be frequently the case, to dislodge a tick, especially Ixodid, with its capitulum intact.

It is evidently due to the belief that the chelicerae function as cutting organs that they have been regarded by a number of workers (Railliet, 1895; Macqueen, 1905; Braun, 1915; Chandler, 1918; Rivas, 1920; Brumpt, 1927) as

the homologue of the mandibles of blood-sucking insects. A consideration of their position in relation to the other appendages of the capitulum, however, can leave little room for doubt that this by no means is the case, for, unlike what is observed in the mandibles of insects, the basal portions of the chelicerae (Fig. 1, *b.ch.*) project backwards through the capitular foramen (*cap. for.*) and overlie the pharynx (*ph.*), so that in this respect they present a much greater resemblance to the labrum-epipharynx than to the mandibles of insects.

While the chelicerae are thus comparable to the labrum-epipharynx, the hypostome presents a similarity to the labium in that it represents no more than a median extension of the ventral portion of the basis capituli. Its tip is conspicuously flattened and rounded, and this alone would lead one to the conclusion that it is ill-adapted for penetrating into the tissues of the host[1]. The presence of a median gutter-like depression on its dorsal surface has been held to furnish some evidence that the hypostome essentially functions as a suctorial organ, the gutter being posteriorly continued into the pharyngeal opening. As will appear, however, from the following account of the sucking apparatus in *O. papillipes*, the hypostomal gutter fulfils a very different function and is entirely unconnected with the pharyngeal tube.

IV. THE SUCKING APPARATUS IN *O. PAPILLIPES*

It would be of interest at this stage briefly to allude to the circumstances under which the writer first came to entertain a doubt as to the correctness of the view hitherto held concerning the feeding mechanism in ticks, for these circumstances happen to have a direct bearing on the morphology of their sucking apparatus which it is the main purpose of the present paper to describe.

Whilst studying the anatomy of *O. papillipes*, I observed that pressure with the dissecting needle on the external surface of the pharynx caused a very noticeable movement in a ridge-like structure overlying the dorsal gutter of the hypostome. On close examination, the ridge proved to be a stylet situated between the chelicerae and the hypostome (Fig. 2). After laborious dissection, the stylet was found to be a tubular process directly continuous with the pharyngeal tube, the intervening buccal chamber being entirely separate from the surrounding exoskeleton, except that it was deeply buried in the hollow of the basal portion of the hypostome (Fig. 3). By gradual traction, the buccal chamber was removed from the hollow of the hypostome (Fig. 4), so that the entire structure, from the distal part of the stylet to the proximal end of the pharynx, could be lifted out of the surrounding debris and after due dehydration mounted on a slide (Fig. 5). A detailed account of the structure of the stylet, of the buccal chamber, and the pharynx, is given below, for together they must be regarded as constituting the sucking apparatus.

[1] It is very sharply pointed, however, in some Ixodidae and larval stages of Argasidae and is well adapted for entering the hole in the skin made by the cutting digits of the chelicerae. The hypostome is an essential organ for anchoring a tick to its host.—Ed.

The sucking stylet

A noticeable feature of the sucking stylet is its remarkably refractile character, and, in the course of numerous attempts, only in one instance did the writer succeed in differentiating it in opaque specimens. A number of staining reagents were used—all applied by means of a minute plug of cotton-wool held with a pair of fine pincers—in order to bring the stylet into prominence, but without success. It is, however, hoped that it will be possible by further trials to devise a suitable method of differentiating the structure.

The stylet (Fig. 3, *st.*) is spatulate distally, but under a low magnification it appears more or less pointed (Fig. 2, *st.*). It has a distinct distal orifice (*o.st.*) and a clearly visible lumen (*l.st.*) running along its entire course and opening into the buccal chamber. It is supported ventrally by a stout chitinous rod, which in cross-sections may be seen as a roundish body staining deeply with Delafield's haematoxylin (Fig. 6, *r.st.*). Compared with the thickness of its walls, the lumen of the stylet is of a fairly wide bore, and in transverse sections of the capitulum the stylet appears as a more or less triangular structure with its vertex pointing downwards and with its base depressed in the middle (Fig. 6, *st.*). It is of interest that a very similar structure was encountered by Robinson and Davidson in cross-sections of the capitulum of *Argas persicus*, being described as "a tongue-like process which covers the pharyngeal orifice", its function being regarded by them to be "to close the pharyngeal opening while the salivary secretion is being forced directly into the wound made by the

---

Legends to figs. 2–11.

O. papillipes.

Fig. 2. The capitulum, showing the relationship of the sucking stylet with the other appendages. ×130.

Fig. 3. The sucking apparatus *in situ*, with a portion of the ventral wall of the basis capituli. ×240.

Fig. 4. The capitulum with the sucking apparatus partially removed. Only one palp is shown and the sucking stylet made to pierce through the wall of the hypostome. ×240.

Fig. 5. The sucking apparatus as completely removed and mounted. A portion of the distal part of the stylet got broken off in this particular specimen. Note chitinous ring encircling margin of the expanded base of stylet. The dotted lines indicate the ventro-lateral margins of the buccal wall. ×240.

Fig. 6. Transverse section of capitulum passing through its middle portion. ×240.

Fig. 7. Transverse section of capitulum passing through anterior part of basis capituli. ×240.

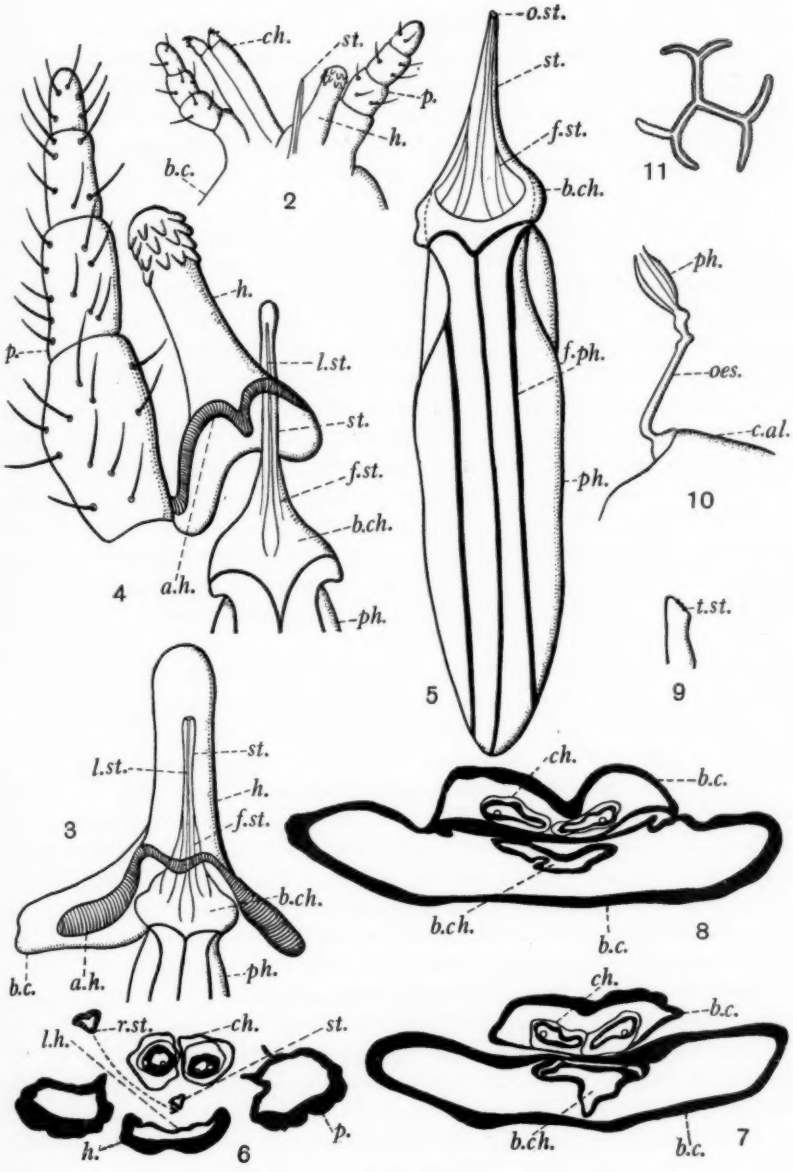
Fig. 8. Transverse section of capitulum at a level posterior to that represented in Fig. 7. ×240.

Fig. 9. Distal extremity of sucking stylet showing a group of three denticles. ×800.

Fig. 10. The pharynx as seen in fresh dissections in normal salt solution. ×40.

Fig. 11. Transverse section passing through middle portion of pharynx. Note the deep infolding of the pharyngeal walls. ×300.

a.h. arch of hypostome; *b.c.* basis capituli; *b.ch.* buccal chamber; *ch.* chelicera; *c.al.* alimentary caeca; *f.ph.* fold of pharyngeal wall; *f.st.* fold of investing membrane of stylet; *h.* hypostome; *l.h.* dorsal lamina of hypostome; *l.st.* lumen of sucking stylet; *oes.* oesophagus; *o.st.* distal orifice of stylet; *p.* palp; *ph.* pharynx; *r.st.* supporting rod of stylet; *st.* stylet; *t.st.* denticles on tip of stylet.


ch.
st.
p.
h.
b.c.
2
o.st.
st.
f.st.
b.ch.
11
ph.
oes.
c.al.
10
p.
h.
l.st.
st.
f.st.
b.ch.
4
a.h.
ph.
f.ph.
ph.
5
t.st.
9
l.st.
st.
h.
f.st.
3
b.ch.
b.c.
a.h.
ph.
ch.
b.c.
b.ch.
b.c.
8
l.h.
r.st.
ch.
st.
h.
6
p.
ch.
b.c.
b.ch.
b.c.
7

Figs. 2–11.

proboscis of the tick". In their figure of an optical section of what they regarded as the buccal cavity, the tongue-like structure is shown as a pointed organ extending distally only a little beyond the base of the hypostome. The failure to obtain a complete longitudinal section of the structure was evidently due to the fact that during the process of dehydration and embedding the stylet is very liable to become displaced from its normal position, so that it is largely a matter of chance to get the razor blade to cut along the entire length of its long axis.

Basally the stylet is conspicuously expanded, its sides being reinforced by what would appear to be the folds of an investing membrane (Fig. 3, *f.st.*). The precise origin of the membrane is obscure, but in one of the preparations (Fig. 5) it seemed to be a continuation of a portion of the buccal wall. The number of these folds varies and they disappear distally, merging into the sides of the stylet. Where the stylet opens into the buccal cavity, it is encircled by a rim of thick chitin (Fig. 5), so that the whole stylet may be likened to the horn of a gramophone placed on its broad end. It is noteworthy that the long axis of the stylet is not in a line with that of the buccal chamber but is considerably inclined, probably to the extent of about 45°.

On the margin of its distal orifice, the stylet bears a number of minute denticles, and under the oil-immersion objective three comparatively large denticles can be counted (Fig. 9), but from the jagged appearance of the rim of the orifice, one would infer that the actual number of denticles is considerably more.

A characteristic property of the stylet is its resistance to the action of water-soluble eosin which so conspicuously stains other chitinous structures. In this respect, it would appear to present a similarity to the shafts of the chelicerae, for it has been observed that, while the cheliceral sheaths take a deep stain with eosin, the shafts themselves are much less affected. This would seem to suggest the possibility that the substance of these structures is not quite the same as that of chitin generally. Microscopically, the stylet, as well as the shafts, presents a keratinous appearance, being very lightly pigmented. The stylet, in particular, appears as a more or less colourless body which can be more clearly made out in a subdued light under the microscope.

The relation of the sucking stylet to the hypostome and the chelicerae is of considerable interest. Dorsally the hypostome is provided with a membranous lamina (Fig. 6, *l.h.*) which evidently protects the stylet against injury coming from the ventral side of the capitulum, whilst the chelicerae situated dorsally to the hypostome with its gutter provide the stylet with a safe resting place.

In a tick that had been fed a few days previously on a fowl, small particles of blood detritus were found scattered over the inner wall of its stylet tube, the buccal chamber, and pharynx. This observation gave conclusive evidence regarding the function of these several parts of the suctorial apparatus.

The buccal chamber

In the literature on ticks the existence of a buccal chamber, as representing an entity distinct from the exoskeleton, has never been recognised. *A priori*, there would seem to be little valid reason for supposing that the alimentary canal, which appears as a discrete structure throughout its course, should lose its identity in the region of the buccal cavity and merge completely in the exoskeletal walls.

According to Robinson and Davidson, the buccal cavity of *A. persicus* is formed by the space enclosed between the basal portion of the lateral margins of the hypostome and "the ventro-lateral walls of that part of the basis capituli which is continued forwards above the mouth to form the cheliceral sheaths". Patton and Cragg, on the other hand, divide the buccal cavity into two parts, of which the "posterior part is closed at all times", whilst the anterior part is "a flat tube, the dorsal wall of which is formed by the apposition of the two mandibles and their sheaths, the ventral by the hypostome". In other words, what has been described by Robinson and Davidson as the buccal *canal* was regarded by Patton and Cragg as forming a part of the buccal *cavity*. The views of the authors cited are therefore somewhat divergent, this being attributable to the difficulty of making out these complicated structures.

In specimens of *Ornithodorus papillipes* freshly dissected out in normal salt solution, the buccal chamber may be seen as a capacious chamber almost completely filling the hollow of the dorsal side of the basal portion of the hypostome (Fig. 3, *b.ch.*). On account of its delicate structure and the thinness of its walls, it is liable to vary somewhat in shape in the process of manipulation, although its general outline is, as a rule, maintained. In its lateral aspect, it is broadly rounded (Fig. 5) and folded dorso-ventrally, so that, when focused at different levels under the microscope, it shows a double margin, thus indicating that it is a hollow organ. Anteriorly, it is firmly apposed against a very thick, conspicuous arch-like structure which forms the basal limit of the dorsal surface of the hypostome (Figs. 3 and 4, *a.h.*). Posteriorly, it projects for a short distance over the dorsal wall of the pharynx, so that the latter actually opens on the postero-ventral side of the buccal chamber. In the region of the antero-lateral angles of the basis capituli, on which the palps are borne, a chitinous extension of the exoskeleton presses against the marginal walls of the buccal chamber with a degree of firmness which sometimes makes it very difficult to detach the chamber intact. After many trials, the best way of removing this organ was found to consist in passing a fine needle underneath its ventral surface, from side to side, and working the needle gently upwards, keeping it all the time firmly held against the internal surface of the ventral wall of the basis capituli.

In cross-sections through its anterior part, the buccal chamber appears as a triangular structure with its apex pointing downwards and with its base almost in contact with the dorsal wall of the basis capituli (Fig. 7, *b.ch.*).

Toward the posterior part, it progressively loses its triangular form, with an increasing amount of space between it and the dorsal wall of the basis capituli (Fig. 8, *b.ch.*).

The pharynx

The morphology of the pharynx has been fully described by Robinson and Davidson in their account of the anatomy of *Argas persicus* and their description applies in all essentials to the pharynx of *Ornithodorus papillipes*. As, however, no picture of the sucking apparatus in ticks can be regarded as complete without some mention of this organ, it will be briefly dealt with in this section. A more important reason for including a description of this organ is the lack of adequate information concerning its morphology when viewed in fresh dissections, almost all published accounts being based upon sections of specimens that had been insufficiently cleared of adherent debris, thus obscuring some of the finer morphological details.

As in Argasid ticks generally, the pharynx of *O. papillipes* is torpedo-shaped, tapering at both extremities and markedly attenuated at its posterior end where it communicates with the oesophagus (Fig. 10). Owing to the stout chitinous lining of its walls, the pharynx will stand continued manipulation with a fine needle, thereby making it possible to view it in all its aspects. It is then seen that its walls are thrown into a number of deep folds, with sharp projecting ridges disposed longitudinally. When focused at different levels, its dorsal and ventral walls may be seen to be continuous with those of the buccal chamber (Fig. 5), thereby giving the latter the appearance of possessing a double margin, as already mentioned.

The appearance of the pharynx in cross-sections has been aptly described by Robinson and Davidson as "triradiate", the extremity of each radius being further divided by an infolding of the pharyngeal wall. In the case of *O. papillipes*, however, the great depth of the pharyngeal folds (Fig. 11) presents a striking contrast to that of *Argas persicus*, as figured by Robinson and Davidson, and it would be of interest to determine any possible significance of this contrast in relation to the pumping capacity of the pharynx, as indicated by the duration of the feed in the different species of ticks, for it seems reasonable to suppose that the time occupied in feeding is inversely proportional to the pumping capacity of this organ, although the strength of the sucking stylet itself will have to be considered in coming to a definite conclusion in this respect. An inquiry into this question may furnish some explanation for the great length of time taken by most species of Ixodid ticks to complete a feed, in spite of the heavy armature of their capitulum, as compared with what one observes in the quick-feeding Argasid ticks.

The precise disposition of the pharyngeal folds (*f.ph.*) at their anterior extremities is extremely difficult to determine, for it is seldom that one succeeds in obtaining longitudinal sections of the organ without a certain amount of overlapping of the folds. It would appear, however, that the fold which forms the ventral part of the pharynx is continued to the walls of the

buccal chamber, while the two folds forming the dorso-lateral parts of the organ "loop down" at the level of the pharyngeal opening and meet together at their extremities (Figs. 3, 4, 5). The backwardly directed loop-like termination of the dorso-lateral folds is liable to lead one to the conclusion that at this part they act as a valve to prevent the regurgitation of ingested blood, but a careful examination of their relationship with the more posterior part of the folds shows that this is not the case. Actually, in spite of repeated attempts, the writer has not been able to discover any structure suggestive of a valvular opening and his observation in this respect agrees with that of Robinson and Davidson.

V. THE SUCKING APPARATUS IN IXODID TICKS

A sequel to the finding of the sucking organ in *Ornithodorus papillipes* was an attempt to discover a similar structure among members of the family of Ixodidae. Unfortunately, living examples of any species of this family were not available at the moment, but through the kindness of Prof. Nuttall, the writer was provided with a large number of preserved specimens of *Amblyomma hebraeum*, *A. gemma* and *Rhipicephalus evertsi*. Small lots of these were soaked in a 10 per cent. caustic potash solution for about 6 days, washed with several changes of water and the capitulum carefully examined in the hope that, as in the case of *Ornithodorus papillipes*, the stylet would be found located on the dorsal surface of the hypostome, but all attempts to differentiate it in this manner proved unsuccessful. The whole of the capitulum was then teased out into longitudinal shreds, minutely examined and the stylet found in all the three species as a long filamentous process buried within the longitudinal cleft which characterises the hypostome of most species of Ixodid ticks (Fig. 12). Owing to the specimens being old, the stylet presented a deformed and lacerated appearance, so that a detailed account of its morphology must be postponed until freshly killed material is available. Nevertheless, the essential features of the stylet could be made out with a fair degree of certainty. As shown in Figs. 12 and 13, the stylet is broad distally, has a distinct lumen (*l.st.*) and bears a group of denticles terminally (Fig. 14). A fortunate accident rendered it unnecessary to obtain cross-sections of the stylet in order to demonstrate its tubular character: in the case of a specimen of *Amblyomma hebraeum*, while the stylet was being liberated from the hypostomal cleft with the help of a needle, it got torn asunder at its distal part, thereby exposing a portion of its tubular interior (Fig. 13).

A curious structure encountered in both *Amblyomma* species was a series of tooth-like processes, arranged in rows, at the posterior part of the buccal cavity (Figs. 12 and 15, *t.b.cav.*). A similar structure was also noticed in *Rhipicephalus evertsi*, which, in addition, had a remarkable pair of extremely fine filamentous processes, dentate at their anterior extremities, placed on either side of the sucking stylet. Numerous attempts were made to recover

these processes intact but without success. It is, however, proposed to repeat these attempts with fresh specimens, and if successful, to give a detailed account of their morphology in a future communication.

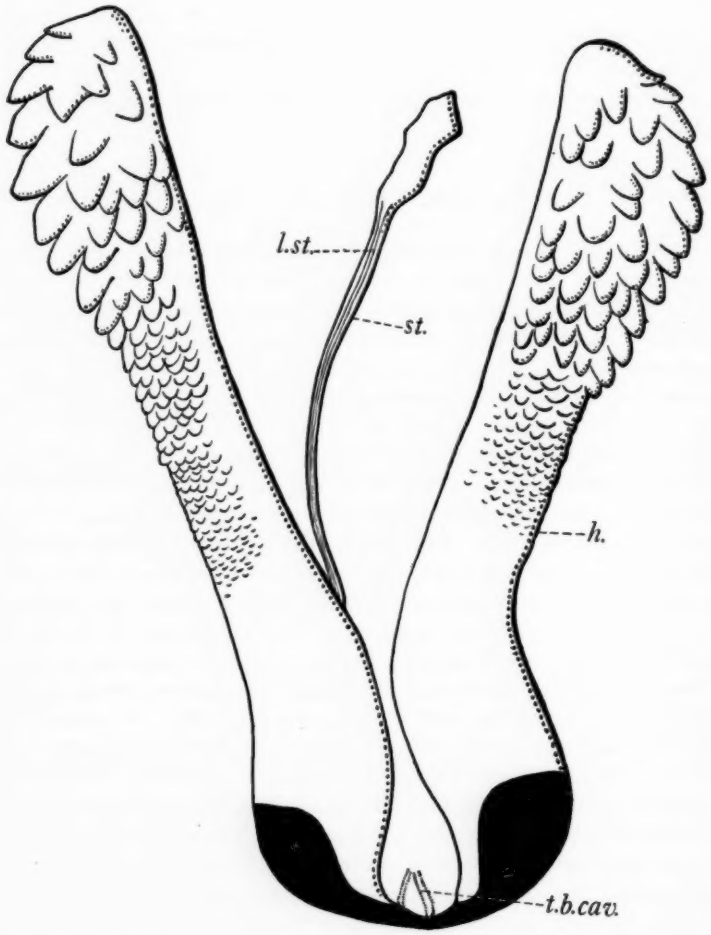
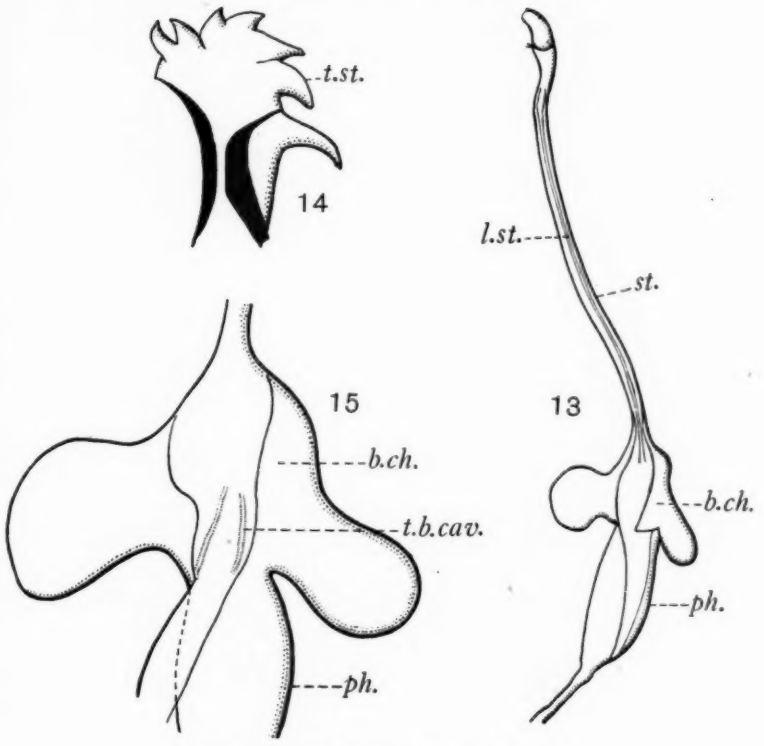


Fig. 12. *A. gemma.* The hypopharynx with its sides separated to expose the sucking stylet. The dark basal portion indicates a piece of adherent chitin. *h.* hypostome; *l.st.* lumen of sucking stylet; *st.* sucking stylet; *t.b.cav.* tooth-like processes in buccal cavity. ×240.



A. hebraeum. (Lettering as in other figs.)

Fig. 13. The sucking apparatus as completely removed and mounted. Note lacerated anterior extremity of stylet, with the tubular interior exposed. ×80.

Fig. 14. A group of denticles on distal extremity of sucking stylet, in the specimen represented in Fig. 13. ×1050.

Fig. 15. The buccal chamber enlarged to show the tooth-like processes (*t.b.cav.*). ×240.

REFERENCES.

BRAUN, MAX (1915). *Die Tierischen Parasiten des Menschen*, p. 387. Von Curt Kabitzsch, Würzburg.

BRUMPT, E. (1927). *Précis de Parasitologie*, p. 857. Mason et Cie, Éditeurs, Paris.

CHANDLER, ASA C. (1918). *Animal Parasites and Human Disease*, p. 354. John Wiley and Sons, Inc., New York.

ELTRINGHAM, H. (1930). *Histological and Illustrative Methods for Entomologists*, pp. 93–4. Clarendon Press, Oxford.

MACQUEEN, J. (1905). *A Treatise on the Parasites and Parasitic Diseases of Domesticated Animals*, p. 83. Baillière, Tindall and Cox, London.

NUTTALL, G. H. F., COOPER, W. F., and ROBINSON, L. E. (1908). The structure and biology of *Haemaphysalis punctata* Can. and Fan. *Parasitology*, **1**, 152–81.

PATTON, W. S. and CRAGG, F. W. (1913). *A Textbook of Medical Entomology*, p. 659. Christian Literature Society for India, Lond., Madras and Calcutta.

RAILLIET, A. (1895). *Traité de Zoologie Médicale et Agricole*, p. 703. Azzelin et Houzeau, Paris.

RIVAS, D. (1920). *Human Parasitology*, p. 482. W. B. Saunders Company, Philadelphia and London.

ROBINSON, L. E., and DAVIDSON, J. (1913–14). The anatomy of *Argas persicus* (Oken 1818). *Parasitology*, **6**, 20–48, 217–256, 382–424.

SHARIF, M. (1928). A revision of the Indian Ixodidae with special reference to the collection in the Indian Museum. *Rec. Ind. Mus.*, **30**, 217–344.

(*MS. received for publication* 22. XII. 34.—Ed.)

ON A NEW COCCIDIAN, *EIMERIA LAMINATA* N.SP., FROM THE INTESTINE OF AN INDIAN TOAD, *BUFO MELANOSTICTUS* SCHNEID.

By HARENDRANATH RAY, M.Sc. (Cal.), Ph.D. (Lond.)

From the Department of Zoology, University College of Science and Technology, Calcutta

(With Plate X, containing Figs. 1–11)

Introduction

In March 1932, while examining the gut contents of *Bufo melanostictus*, I came across a species of coccidian in two specimens. On consulting the literature, I found that while five species of *Eimeria* have been recorded from the intestinal region of anurous amphibians, none of them corresponds with the one I have observed. Moreover, I notice that for no one of these five species has the whole life history been described.[1]

I think it of interest, therefore, to give the following detailed account of the first coccidian recorded from an Indian toad. While it is true that of the 200 specimens of *Bufo melanostictus* that I examined for the coccidian only two were infected,[2] by good fortune it happened that schizogony and sporogony were occurring simultaneously in the same individual, and so I was able to get all essential information from this limited material.[3]

The parasite from *Bufo melanostictus* is a species of *Eimeria*, but it shows a number of peculiar characteristics; among these I may mention the paired flanges or laminae at the anterior end of the body, and the name I propose to give to the new species, *E. laminata*, refers to this structural feature.

Methods

The living parasite was studied in sealed preparations of the rectal and intestinal contents diluted with saline. In such conditions it remained alive and very active for over 3 hours; after that all stages but the oocysts became plasmolysed.

[1] *Eimeria ranarum* (Labbé), *E.* (*Paracoccidium*) *prévôti* Laveran and Mesnil, *E. ranae* Dobell, *E. neglecta* Nöller, and *E. belawini* Yakimoff. The first of these inhabits the nuclei; stages of the others have been found in the cytoplasm of the epithelial cells or free in the lumen of the gut.

[2] Other Protozoa occurring in these two toads were a ciliate, *Cépèdea* sp., from the rectum, and a myxosporidian, *Cystodiscus* sp., from the gall-bladder.

[3] Heavily infected portions of the intestinal epithelium showed marked signs of degeneration, the cells being reduced to mere frames for the parasites, while their nuclei were either hypertrophied or shrunken. In places whole tracts of epithelium were practically replaced by parasites.

Smears were fixed in Schaudinn's fluid or Bouin-Duboscq-Brasil's fluid, and stained with Delafield's or Heidenhain's haemotoxylin. The second of these fixatives was also used for portions of infected intestine, which was cut in sections 6μ thick.

Observations on the free forms of the Coccidium

In a fresh smear made in normal saline, active "gregarinulae" were seen in large numbers; they ranged in size from $3 \times 1{\cdot}25\mu$ to $12 \times 6\mu$. The small ones were more active than the large. I noticed two types of movement. One was a bowing movement, like that of the gregarine *Selenidium*: the other was a screwing movement, in which the organism turned rapidly on its long axis and advanced with the more pointed end of the body in front. Closer observation showed that on either side of this, presumably the anterior, end is a hyaline blade-like structure, or lamina. I consider that the screwing movement is effected by certain contractile elements which, when fixed and stained, appear as a siderophilous area at the more pointed end.

That this is the anterior end of the parasite is confirmed by studying sections (Fig. 1*b*), and I have often observed individuals with the pointed end apposed to the epithelial lining of the gut. It is perhaps by the help of the screwing movement and the blades that the parasites bore their way into the cells. These little bladed organisms are liberated merozoites or young schizonts.

My study of smears and sections has convinced me that there are two types of schizont, giving rise to male and female parasites respectively. The nucleus of either type begins to divide before the schizont reaches full size, or even before it effects entry into a cell; indeed, nuclear division is to be seen in the smallest forms, free in the gut lumen. It would appear, then, that intracellular habit during early development is not obligatory for this coccidian in its asexual phase.

Microschizogony and the formation of microgametes

In fixed and stained material the schizonts that will give rise to the male parasites can readily be distinguished from the other type by the absence from their cytoplasm of darkly staining granules. Figs. 2 and 3 show the two types; the one marked 3 is destined to form males. In both appear the anterior, hyaline laminae, with a siderophilous area between the blades, where lie what I regard as the contractile elements. The smallest individual that I could identify as a microschizont measured about $6 \times 2\mu$; its body was slightly compressed, and the anterior end was more pointed than the posterior. Some individuals of these dimensions showed signs of nuclear division.

After entry into a cell, the schizont continues to grow to a size of $12 \times 5{\cdot}5\mu$. These full-grown forms usually contain six to eight merozoites arranged approximately parallel to the long axis of the parent (Fig. 4). It is not possible to ascertain whether more than one generation of these is developed; but the abundance of this type of schizogony in the preparations suggests that perhaps this happens.

In any case, the ultimate merozoites here are the male forms of the parasite. As they proceed to form microgametes, each enlarges and becomes either oval or irregular in shape. Early stages of nuclear division were unfortunately very rare in my preparations. The abundant stages measured about $13 \times 9\mu$ and showed numerous nuclei arranged at the periphery. Mitotic figures in late anaphase were sometimes seen, which showed four small curved chromosomes situated near each pole; I believe this to be the haploid number. The cytoplasm round each gamete nucleus becomes raised into a hyaline cone which ultimately is converted into the flagellum (Fig. 8). While the furrows round the cones are getting deeper, each rounded gamete nucleus elongates and occupies almost the whole base of a cone. The process is completed by further deepening of the furrows, and finally the gametes are pinched off from the parent cytoplasm. A fully formed microgamete measures 2·75–3μ in length and is about 1μ in breadth; at its free end it has a single flagellum of about the same length as its body (Fig. 9).

Macroschizogony and the fate of the macromerozoites

In contrast with the first type of schizont, those that will give rise to female parasites have from a very early stage dark-staining granules scattered through the cytoplasm (Fig. 2). Here also signs of nuclear division appear precociously. A full-grown intracellular schizont is 9–10μ in diameter and contains as many as twenty to thirty merozoites of oval form, one end being slightly pointed. They form a central group surrounded by the deeply staining peripheral cytoplasm of the mother schizont (Fig. 5). Even at this stage the daughter organisms show the beginnings of the characteristic darkly staining granules in their cytoplasm. The number of merozoites formed and the presence of these cytoplasmic inclusions help one to distinguish macroschizogony from microschizogony.

A full-grown female parasite measures about $11 \times 6{\cdot}5\mu$; its cytoplasm shows many irregular inclusions which stain deeply with haematoxylin. The nucleus at this stage is spherical and has a diameter of slightly over 3μ: the nuclear membrane is very thin and there is no staining content other than the karysome (Fig. 6). The nucleus now elongates in a direction at right angles to the long axis of the body, and granules appear in it which seem to give rise to a tangled mass of chromatin threads (Fig. 7). The direction of this "fertilisation spindle" is probably imposed by the persistence of the anterior differentiation of the parasite, which still shows its laminae and the contractile elements.

Sporogony

Sporogony is strictly intracellular. Unfortunately, the actual process of fertilisation could not be seen. A stage like that shown in Fig. 7 was common. In addition to the usual inclusions, the cytoplasm of the zygote shows a deposit of minute refringent granules at the periphery, which at once distinguishes it from an unfertilised female parasite. The next stage found in abund-

ance is the oocyst. The oocyst is spherical and has a diameter of 8–11 μ. Its contents divide to give four sporoblasts; there is no oocystic residuum (Fig. 10). The spindle-shaped spores measure from 4·5 μ to nearly 6 μ in length, with a breadth of slightly under 3 μ; they each contain two sporozoites and a residuum (Fig. 11). Development of the spores and their contents proceeds inside the host cell; the mature oocysts are then dropped into the lumen of the intestine, and pass from the host's body in the faecal matter.

Conclusions

The dizoic tetrasporocystid condition of the oocyst at once places this coccidian in the genus *Eimeria*. The accompanying table compares the species of *Eimeria* from frogs and toads in respect of oocyst and sporocyst. It will be seen that the form and dimensions of these bodies are sufficiently different in the coccidian from India to warrant my considering it a new species: but I base the specific name *laminata* on the peculiar blade-like projections with which the anterior end of the parasite is furnished.

Table giving comparisons of the species of Eimeria *in frogs and toads*

	Oocyst			Spore				
Name	Form	Size in microns	Residuum	Form	Size in microns	Residuum	Locality	Host
E. ranarum (Labbé) 1894	Oval	17×12	0	Fusiform	7×4	+	France	*Rana temporaria* *R. esculenta*
E. prévôti Laveran et Mesnil 1902	Oval	20–22×12–15	+	Oval	—	+	France	*R. esculenta*
E. ranae Dobell 1908	Rounded oval	18–22	+	Naviculoid	14×7	+	England (Cambridge) Germany (Munich)	*R. temporaria* *R. esculenta*
E. neglecta Nöller 1920	Round	9–10	0	Oval	7×4	+	Germany (Hamburg, Thüringia)	*R. temporaria* *R. esculenta*
E. belawini Yakimoff 1930	Round	12·21	0	Round	4·44	?	North Caucasia (Pjatigorsk)	*Hyla arborea*
E. laminata n.sp.	Spherical	8–11	0	Fusiform	4·6–5·8	+	India (Calcutta)	*Bufo melanostictus*

Other features of interest may be summarised as follows: the asexual forms and their young products show remarkable activity: schizogony is initiated at a very early stage: there are two kinds of schizont: (*a*) macroschizonts, releasing twenty to thirty merozoites which develop into macrogametes, and (*b*) microschizonts, producing six to eight merozoites which become males and give rise to numerous uniflagellate microgametes.

REFERENCES

Dobell, C. (1908). On the intestinal protozoan parasites of frogs and toads. *Proc. Camb. phil. Soc.* **14**, 428.

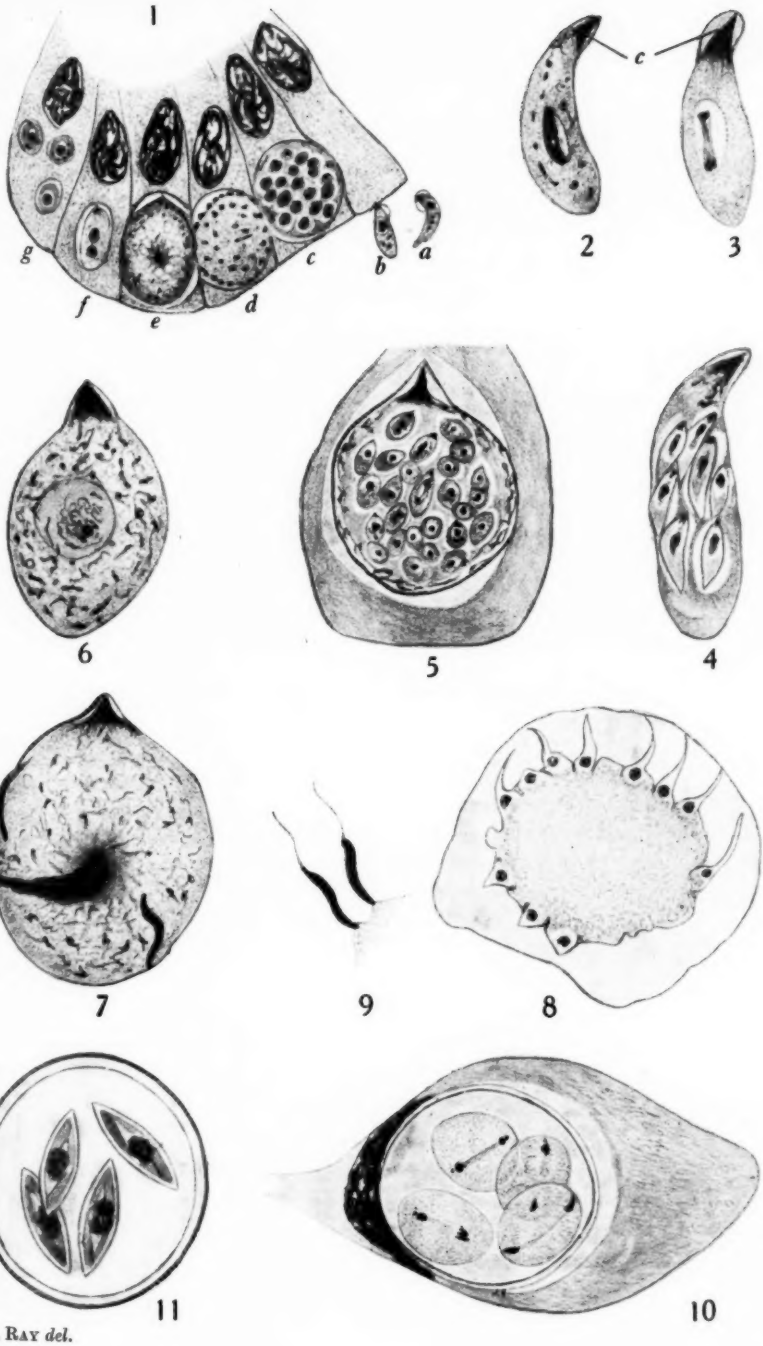
—— (1909). Researches on the intestinal protozoa of frogs and toads. *Quart. J. micr. Sci.* **53**, 201.

Labbé, A. (1894). Recherches sur les parasites endoglobulaires du sang des vertébrés. *Arch. Zool. exp. gén.* (Sér. 3), **2**, 55.

Laveran, A. and Mesnil, F. (1902). Sur deux coccidies intestinales de la *Rana esculenta*. *C.R. Soc. Biol.*, Paris, **54**, 857.

Nöller, W. (1920). Kleine Beobachtungen an parasitischen Protozoen. *Arch. Protistenk.* **41**, 169.

Yakimoff, W. L. (1930). Neue Coccidien der Frösche in Nordkaukasus. *Ibid.* **70**, 639.



H. RAY *del.*

UNIV. OF MICH.

EXPLANATION OF PLATE X

All figures are camera-lucida drawings either of living specimens or from smears or sections fixed in Bouin-Duboscq-Brasil's fluid (unless otherwise stated) and stained with Heidenhain's iron-haematoxylin. ×3500 unless otherwise stated.

Eimeria laminata n.sp.

Fig. 1. From a transverse section of the small intestine. ×1666. *a*, merozoite free in the lumen; *b*, merozoite entering a cell; *c*, macroschizogony; *d*, microgamete formation; *e*, macrogamete; *f*, microschizont; *g*, three merozoites in one cell.

Fig. 2. Macroschizont, from a smear fixed in Schaudinn's fluid. *c*, contractile elements.

Fig. 3. Microschizont, from a smear fixed in Schaudinn's fluid.

Fig. 4. Microschizogony, from a smear.

Fig. 5. Macroschizogony, from a smear.

Fig. 6. Macrogamete, from a section of the intestine (outline of the host cell omitted).

Fig. 7. Macrogamete with the fertilisation spindle situated at right angles to its long axis. Two microgametes are shown lying on its body. From a section (outline of the host cell omitted).

Fig. 8. Microgamete formation, from a section; the host cell represented in outline only.

Fig. 9. Fully formed microgametes, from section.

Fig. 10. Intracellular oocyst showing four sporoblasts, from a section.

Fig. 11. Mature oocyst, from a fresh smear.

(*MS. received for publication* 7. II. 1935.—Ed.)

NEW PARASITES OF THE GENUS *ORIENTOPHORUS*, N.GEN. (FAMILY FELLODISTOMIDAE) FROM AN INDIAN FRESH-WATER FISH, *CLUPEA ILISHA*[1]

By HAR DAYAL SRIVASTAVA, M.Sc.
Lady Tata Research Scholar

Zoology Department, University of Allahabad, India

(With 4 Figures in the Text)

The family Fellodistomidae Nicoll 1913 comprises two subfamilies, Fellodistominae Nicoll 1909 and Haplocladinae Odhner 1911, which are composed of a large number of genera of small flukes, all parasitic in fishes. The type genus of the family was described by Stafford in 1904 from the gall bladder of a Canadian wolf-fish, *Anarrhichas lupus*. Subsequently several new genera have been added from European and American fishes, but until now no representative of the family has been reported from India.

The nomenclature of the family Fellodistomidae Nicoll 1913 or Steringophoridae Odhner 1911, has been discussed by Odhner (1911), Nicoll (1913), Poche (1925), and Stunkard and Nigrelli (1930). The last-named authors have discussed at considerable length the untenability of the name Steringophoridae and have quoted from a personal communication to them the opinion of Prof. Stiles on the question. This opinion is so decisive that the adoption of the name Fellodistomidae becomes necessary.

Orientophorus brevichrus, n.gen. et n.sp.

This species represents the most common fish trematode in winter months in Northern India, when nearly every host is found heavily infected. The intensity of infection varies from 10 to 80 parasites in a single host. This parasite occurs in association with *Lecithaster indicus* Srivastava and *L. extralobus* Srivastava and some new members of the subfamilies Sterrhurinae and Dinurinae, shortly to be described. With the approach of summer, however, the frequency of infection rapidly declines, reaching below 10 per cent. in midsummer. The living worms are thick and non-transparent with very little power of contraction and expansion except in the pre-acetabular region which is very mobile. Outside their natural habitat they can live for hardly more than four hours in any solution. The ovoid body in permanent mounts is 1·3–1·7 long and 0·7–0·9 broad across the region of testes. The pretesticular region is covered with small backwardly pointed spines $0{\cdot}006 \times 0{\cdot}001$.

[1] This paper was read before the Academy of Sciences, U.P., Allahabad, India, on December 12th, 1934. All measurements are in mm.

Unicellular cutaneous glands occur all over the body, especially in the region of the oesophagus.

The oral sucker, 0·15–0·19 in diameter, is anteriorly directed during life,

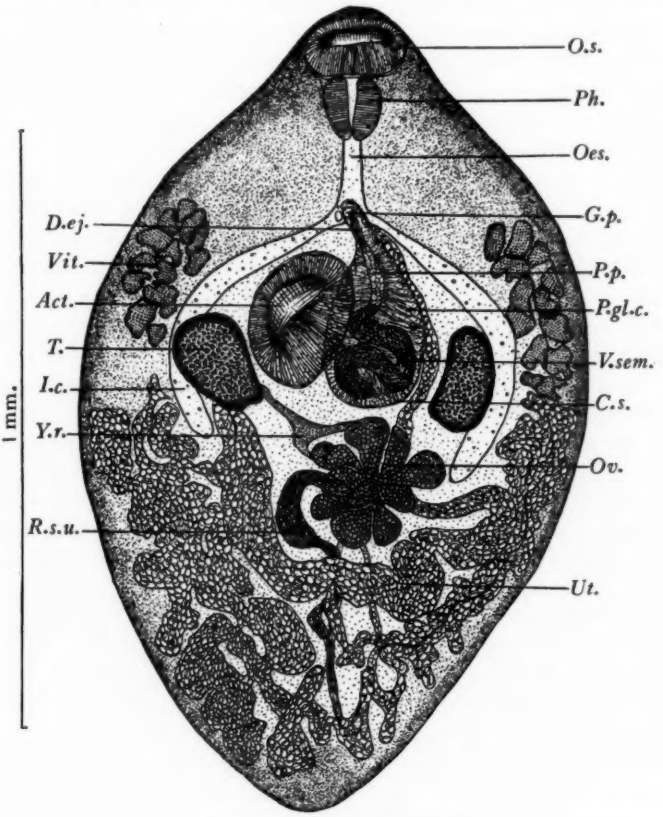


Fig. 1. *Orientophoros brevichrus.*

Lettering:

Act.	Acetabulum.	*P.p.*	Pars prostatica.
C.s.	Cirrus sac.	*Ph.*	Pharynx.
D.ej.	Ductus ejaculatorius.	*R.s.u.*	Receptaculum seminis uterinum.
G.p.	Genital pore.	*T.*	Testis.
I.c.	Intestinal caecum.	*Ut.*	Uterus.
O.s.	Oral sucker.	*V.sem.*	Vesicula seminalis.
Oes.	Oesophagus.	*Vit.*	Vitellaria.
Ov.	Ovary.	*Y.r.*	Yolk reservoir.
P.gl.c.	Prostate gland cells.		

but in fixed specimens it comes to occupy a subterminal position on the ventral surface. The acetabulum, 0·14–0·25 diameter, is situated in the median line, sometimes slightly to one side close behind anterior third body length. The pre-

pharynx is very small, visible in sections only; the pharynx is well developed and oval, 0·1–0·12 × 0·09–0·11. The oesophagus, 0·12–0·2, bifurcates at about the end of anterior third of body into two moderately long caeca which diverge out into a semicircle enclosing the genital organs. Posteriorly the caeca terminate blindly at about the middle of ovary.

The testes, two in number, are compact structures with an oval or reniform outline, 0·18–0·21 × 0·12. They are situated symmetrically, one on each side, in the intercaecal space in the middle third of body, sometimes partially overlapped by the intestinal caeca. The cirrus sac, 0·31–0·36 × 0·17–0·21, is flask-shaped with a short neck, situated to the left side of the acetabulum between the latter and the left testis, extending from the intestinal bifurcation to the level of posterior end of testes. It encloses an **S**-shaped vesicula seminalis 0·31–0·41 long, and a straight pars prostatica 0·09 × 0·02, which continues anteriorly into a short ductus ejaculatorius 0·05 long and a knob-like cirrus. The remaining free space in the cirrus sac is filled with highly developed prostate gland cells. The male and female openings lie separately in a small, shallow and eversible genital atrium situated ventrally at the level of the intestinal bifurcation.

The ovary, 0·19–0·31 in diameter, is situated close behind the anterior half of body, slightly to the left side. It is composed of three main lobes each of which is further divided into three or more lobes. In some specimens the central connections of the lobes become so faint as to give the ovary a follicular appearance. The oviduct arises dorsally from the centre of the ovary and immediately before entering the ootype gives off the Laurer's canal and then receives the common vitelline duct. The shell gland cells form a diffuse mass.

The vitelline glands, composed of small irregular follicles, lie laterally between the caeca and the body wall, extending from the level of the intestinal bifurcation to the middle of testes. The transverse vitelline duct of each side runs obliquely towards the median line and unites with its fellow of the other side in level with the anterior margin of ovary to form a triangular yolk reservoir from the posterior end of which is given off the common vitelline duct which runs to the left side to open into the ootype.

The receptaculum seminis is absent. The initial part of the uterus as it emerges out of the ootype dilates to form a distinct receptaculum seminis uterinum. The uterus is enormously developed and lies in irregularly disposed longitudinal and transverse coils, sometimes overlapping the ovary, extending from behind the vitellaria to the hinder end of body. The terminal outgoing part of the uterus lies between the cirrus sac and the left testis and passes into a feebly developed metraterm. The eggs are operculate and oval, 0·013–0·02 × 0·01–0·012.

The excretory bladder is **V**-shaped, the arms reaching anteriorly as far forward as the pharynx. Posteriorly the two cornua unite to form a very small median stem 0·07 long, which is surrounded at its end by a spherical sphincter 0·05 diameter. The bladder opens at the posterior tip of the body.

Orientophorus gangeticus n.sp.

Body thick, ovoid with broadly rounded off ends, spinose up to the region of ovary, spines minute, backwardly directed. Mature specimens are 1·34–1·65 long and 0·79–0·97 broad across the level of testes. Unicellular glands occur all over the body. Suckers powerful, spherical and equal in size; oral sucker terminal, 0·17–0·18 diameter; acetabulum, 0·17–0·2 diameter, situated

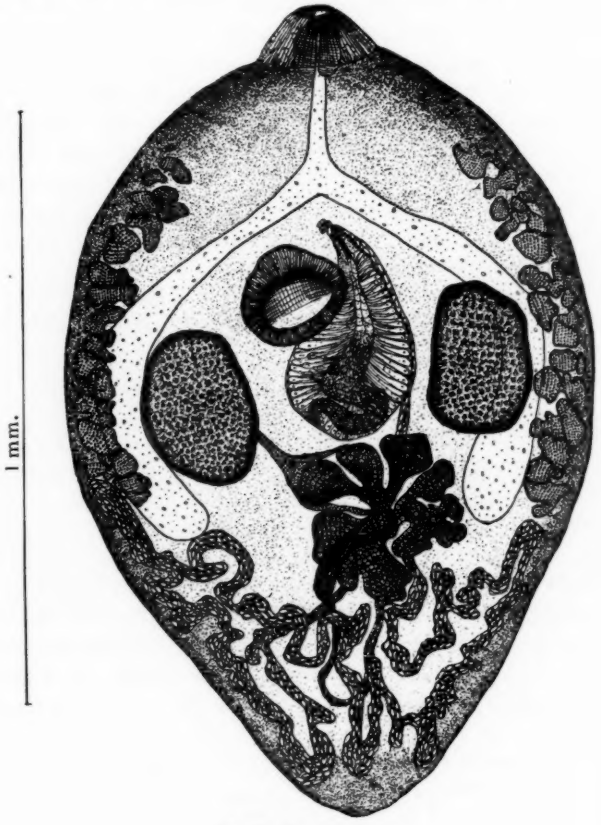


Fig. 2. *O. gangeticus.*

close behind anterior third body length. Prepharynx rudimentary; pharynx large, pyriform, 0·11–0·15 × 0·08–0·12; oesophagus 0·16–0·18 long; caeca moderately long and broad, diverging out into an arc to enclose the genitalia and terminating posteriorly in front of posterior third body length. Genital atrium small, situated half way between intestinal bifurcation and ventral sucker. Excretory bladder as in the type species.

Testes compact, massive, 0·2–0·34 × 0·12–0·2, situated asymmetrically, one

on each side, in the intercaecal space; left testis slightly larger and cephalad, at times overlapped by the caecum of the same side. Cirrus sac large, 0·36–0·4 × 0·17–0·2, nearly twice as long as broad, situated to the left of acetabulum between the latter and left testis and extending from genital atrium to anterior border of ovary. Seminal vesicle roughly **S**-shaped, 0·34–0·4 long; pars prostatica straight, tubular, 0·15–0·17 long, followed by a small ductus ejaculatorius and terminating into a knob-like cirrus; prostate glands well developed, filling all the available space in the cirrus sac. Ovary deeply lobed, 0·26–0·28 × 0·24–0·26, slightly to left side close behind cirrus sac. Shell gland complex dorsal to ovary; Laurer's canal present; seminal receptacle lacking. Early coils of uterus filled with sperms forming a distinct receptaculum seminis uterinum; uterus intricately coiled, extending from vitellaria to hinder end of body, sometimes overlapping ovary; passing terminally between cirrus sac and left testis; metraterm feebly muscular; eggs oval, operculate, 0·016 0·025 × 0·012. Vitellaria follicular, lateral, extracaecal, extending from posterior third of oesophagus to blind ends of caeca; yolk reservoir triangular, median between ovary and cirrus sac.

This species differs from the type species in the larger extent of vitellaria and the position of the genital opening; besides there are differences in the size of the various organs.

Orientophorus ilishii n.sp.

Colour deep brown; body plump, tapering posteriorly; uniformly spinose, spines minute, backwardly directed; mature specimens in permanent mounts measure 0·75–1·04 long and 0·62–0·71 across the level of testes. Unicellular glands present as in other species. Suckers well developed; oral sucker terminal, broader than long, 0·1–0·12 × 0·12–0·14 in size; acetabulum spherical, 0·14–0·15 in diameter, situated just behind first third body length; prepharynx extremely small; pharynx oval, elongated, 0·11–0·13 × 0·067–0·007; oesophagus absent; caeca broad diverging out into a semicircle to enclose the genitalia and ending blindly at about middle of ovary. Genital atrium small and shallow, situated immediately behind intestinal bifurcation. Excretory bladder as in type species.

Testes two, regular, situated almost symmetrically, one on each side of acetabulum, in the intercaecal space partly overlapping intestinal caeca; right testis, 0·28–0·3 × 0·12, is larger than the left which is 0·17–0·2 × 0·15; cirrus sac oval, 0·29–0·31 × 0·17, lying to the right of acetabulum between the latter and right testis, extending posteriorly to level of hinder margin of left testis; seminal vesicle sinuous, pars prostatica and ductus ejaculatorius short, cirrus small. Ovary deeply lobed 0·17–0·2 × 0·19, situated slightly to the left side behind testis. Shell gland complex dorsal to ovary; Laurer's canal present; receptaculum seminis absent; receptaculum seminis uterinum present; uterine coils fill up post-testicular space, often overlapping ovary; terminal part of uterus passes to left of acetabulum; metraterm poorly developed. Eggs oval,

operculate, 0·02 × 0·012. Vitellaria follicular, lateral, partly overlapping caeca, extending from level of anterior border of pharynx to that of anterior margin of ovary; yolk reservoir large, triangular, median and in level with anterior half of ovary.

This species differs from the last two species in the absence of oesophagus, extent of vitellaria, shape and size of body and cirrus sac, in addition to minute differences in the size of the various organs.

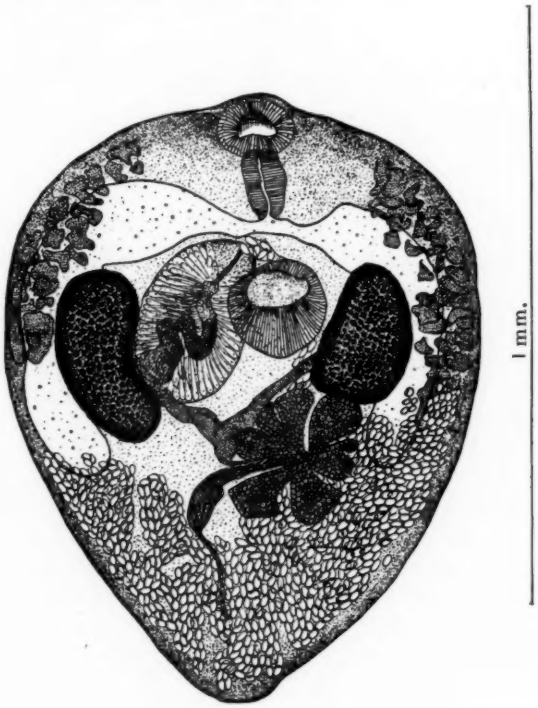


Fig. 3. *O. ilishii.*

Orientophorus clupii n.sp.

Body elongated oval with bluntly pointed ends, pre-acetabular region studded with minute backwardly directed spines. Adult specimens measure 1·3–1·5 long and 0·7–0·8 across the level of testes. As in other species unicellular glands occur all over the body, especially in the region of oesophagus. Suckers broader than long; oral sucker subterminal, 0·1–0·12 × 0·13–0·15 diameter; acetabulum slightly larger than oral sucker, 0·14–0·16 × 0·16–0·17 diameter, situated a little behind first third of body. Prepharynx very small; pharynx oval, 0·17 × 0·09; oesophagus 0·08–0·1 long; caeca fairly long ending

blindly in level with posterior border of ovary. Genital atrium small and shallow, situated at posterior end of pharynx. Excretory bladder as in type species.

Testes oval, situated symmetrically in the intercaecal space, one on each side of acetabulum, partly overlapping caeca, 0·28 × 0·14. Cirrus sac claviform, 0·28 × 0·17 and extending from anterior border of acetabulum to genital atrium; vesicula seminalis **S**-shaped; pars prostatica surrounded by prostate gland cells; short ductus ejaculatorius present; cirrus minute and knob-shaped.

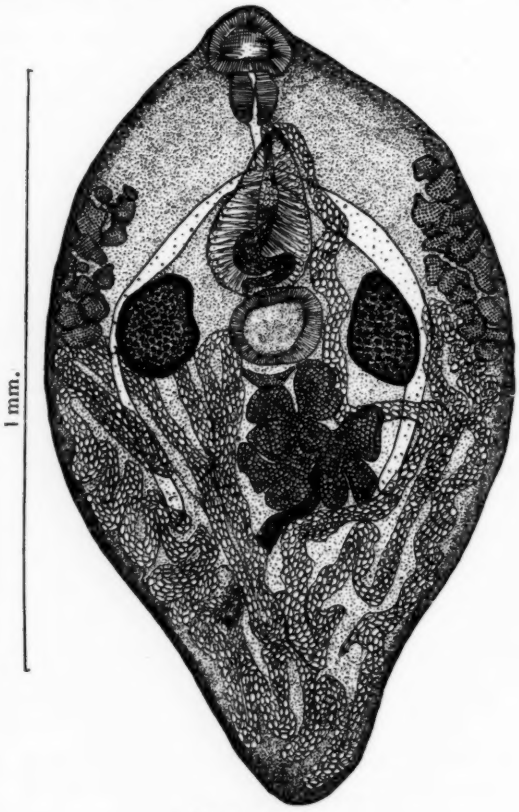


Fig. 4. *O. clupii.*

Ovary deeply lobed, 0·26 diameter, situated a little to the left close behind acetabulum. Shell gland complex dorsal to ovary; Laurer's canal present; receptaculum seminis lacking; receptaculum seminis uterinum present; uterine coils extend from middle of acetabulum to hinder end of body partially overlapping vitellaria, ovary and testes; terminal part of uterus passes between acetabulum and left testis; metraterm feebly muscular; eggs 0·015 × 0·01. Vitellaria follicular, lateral, extracaecal, never overlapping caeca, extending

from level of intestinal bifurcation to middle of testes. A small and inconspicuous yolk reservoir lies just behind the acetabulum.

This species differs from all the previous species in the position of the genital opening and the cirrus sac and the greater length of the intestinal caeca. In the extent of vitellaria it resembles the type species but differs from the latter in the position of the genital pore and cirrus sac and length of intestinal caeca. From *O. ilishii* and *O. gangeticus* it differs in the extent of vitellaria also.

REMARKS ON THE GENUS *ORIENTOPHORUS* n.gen.

The genus *Orientophorus* obviously belongs to the family Fellodistomidae Nicoll and the subfamily Fellodistominae Nicoll, on account of the general topography of the organs, shape of excretory bladder and host. Though the position of the genital pore in *O. clupii* is a departure from the usual condition met with in the family, this species so closely resembles the other three species of the genus in all other features that the aberrant position of the genital opening can only be regarded as a specific character. The post-testicular position of the ovary, however, is characteristic of the genus *Orientophorus*. The ovary in all the genera of the family is pre-testicular except in *Bacciger* Nicoll, where it lies between the testes.

DIAGNOSIS OF THE GENUS *ORIENTOPHORUS* n.gen.

Moderate sized fellodistomids, 0·75–1·7; body oval, thick, spinose, spines minute backwardly directed. Suckers well developed, nearly equal in size; acetabulum situated close behind anterior third of body. Genital pore median, varying in position from in front of acetabulum to posterior end of pharynx. Excretory bladder **V**-shaped with a very small median stem, cornua extending up to pharynx, excretory opening terminal. Testes, two, compact, lateral, intercaecal, situated lateral to acetabulum in middle third of body. Cirrus sac large, oval, enclosing a well developed **S**-shaped vesicula seminalis; pars prostatica surrounded by huge prostate gland cells; ductus ejaculatorius and a small cirrus. Ovary deeply lobed, post-testicular, slightly to one side. Laurer's canal present; shell gland complex dorsal to ovary; receptaculum seminis lacking. Receptaculum seminis uterinum well developed. Uterus enormously developed, extending from middle of acetabulum to hinder end of body; metraterm feebly muscular. Vitellaria follicular, lateral, extracaecal, sometimes overlapping caeca, extending from level of pharynx to hinder end of caeca. Yolk reservoir triangular, median, between acetabulum and ovary. Eggs yellow, oval, operculate, 0·013–0·025 × 0·01–0·012. Host: fresh-water fish, *Clupea ilisha*. Type species: *O. brevichrus*.

This work was carried out under the supervision of Dr H. R. Mehra to whom my respectful thanks are due for valuable help and advice. I am also thankful

to Dr D. R. Bhattacharya for providing facilities in the Department. I am grateful to the Trustees of Lady Tata Memorial Trust, Bombay, for the grant of a scholarship for research in Helminthology.

REFERENCES

LINTON, E. (1900). Fish parasites collected at Wood's Hole in 1898. *Bull. U.S. Fish Comm.* **19**, 267.

—— (1901). Parasites of fishes of the Wood's Hole region. *Ibid.* p. 405.

—— (1905). Parasites of fishes of Beaufort, North Carolina. *Bull. U.S. Bur. Fish.* **24**, 321.

—— (1910). Helminth fauna of the Dry Tortugas. II. Trematodes. *Publ. Carneg. Inst. Wash.* No. 133, 11.

—— (1911). Trematodes of the Dry Tortugas. *Science*, **33**, 303.

MANTER, H. W. (1934). Some digenetic Trematodes from deep-water fish of Tortugas, Florida. *Pap. Tortugas Lab.* **27**. *Publ. Carneg. Inst. Wash.* No. 435, 257.

NICOLL, W. (1909). Studies on the structure and classification of the digenetic Trematodes. *Quart. J. micros. Sci.* **53**, 391.

—— (1913). Trematode parasites from food fishes of the North Sea. *Parasitology*, **6**, 188.

—— (1913). The trematode parasites of fishes from the English Channel. *J. Mar. Biol. Ass. U.K.* N.S. **10**, 466.

ODHNER, T. (1911). I. Wissenchaftliche Mitteilungen. 1. Zum naturlichen System der Digenen Trematoden. III. *Zool. Anz.* **38**, 97.

POCHE, F. (1925). Das System der Platodaria. *Arch. Naturgesch.* Abt. B, **91**, 1.

SRIVASTAVA, HAR DAYAL (1934). New Hemiurids from Indian fresh-water fishes. Part I. *Proc. Acad. Sci., U.P., Allad., India* (in Press).

STAFFORD, J. (1904). Trematodes from Canadian fishes. *Zool. Anz.* **27**, 481.

STUNKARD, H. W. and NIGRELLI, R. F. (1930). On *Distomum vibex* Linton, with special reference to its systematic position. *Biol. Bull. Wood's Hole*, **58**, 336.

(*MS. received for publication* 11. II. 1935.—Ed.)

THE TOXICITY OF BACTERICIDAL SUBSTANCES FOR FLAGELLATE PARASITES, WITH SPECIAL REFERENCE TO THEIR APPLICATION IN THE ISOLATION OF *LEPTOMONAS* FORMS

By FRANK CHARLES HAPPOLD and DORA STEPHENSON

From the Department of Pathology and Bacteriology, The University of Leeds

Introduction

As previously stated by Boxhall, Happold and Lloyd (1934), the cultivation of flagellate parasites, from the alimentary tracts of Diptera, is difficult owing to the general presence of bacteria which rapidly overgrow the cultures. These authors succeeded in isolating two flagellates, one from *Polietes lardaria* and another from a *Calliphora*, using quinanil as a bactericidal substance. The method adopted was as follows: the flagellates were exposed to 1/10,000 or 1/100,000 quinanil for 2–4 or more hours and inoculated into Locke serum agar containing quinanil in concentrations of 1/10,000 or 1/50,000. Since the choice of quinanil was purely arbitrary, it was felt desirable to consider the following extended series of compounds:

Gentian violet.

Brilliant green, a powerful bactericidal agent with weak amoebicidal action.

Acriflavine.

Five anil quinoline derivatives, belonging to a new group of compounds shown by Browning, Cohen, Ellingworth and Gulbransen to possess strong antiseptic and relatively weak trypanocidal properties.

(1) **48s**. Quinanil, the compound 2 (*p*-dimethylaminoanil)-6-methylquinoline methochloride, described by Browning, Cohen, Ellingworth and Gulbransen (1926), which has undergone a process of purification. According to Armitage *et al.* (1929) this substance has a relatively high antiseptic titre, together with a low toxicity and its action is only slightly diminished by the presence of serum.

The following three compounds first described by Browning, Cohen, Ellingworth and Gulbransen (1926):

(2) **52**. 2 (*p*-dimethylaminoanil)-β-naphthoquinoline methochloride.

(3) **59**. 2 (*p*-dimethylaminoanil)-6-acetylaminoquinoline methochloride.

(4) **61**. The methosulphate of 59.

(5) **113**. The condensed product of *p*-nitrosomethyltetrahydroquinoline and 6-acetylaminoquinoline methochloride, described by Browning, Cohen, Ellingworth and Gulbransen (1928).

It has been our aim not only to determine the lethal concentrations of these compounds for *Staphylococcus aureus*, *Bacillus coli*, *B. prodigiosus*, nine insect

and plant flagellates and four strains of *Leishmania*, after varying periods of exposure and in the absence of media, but also to determine the limiting concentrations of these reagents which would permit growth in suitable media. It was thereby hoped that other compounds, which could be used in the admittedly difficult work of isolation of further insect parasites, might be found.

Material and methods

Nine strains of insect and plant flagellates and four *Leishmania* strains were used in our experiments. Following Wenyon (1926) we shall use the generic term *Leptomonas* rather than *Herpetomonas* for the former group, since no trypanosome forms have been demonstrated in our cultures. The following strains were obtained through the courtesy of Dr St John-Brooks from the National Collection of Type Cultures:

Flagellate	Source	
Leptomonas oncopelti 2	*Oncopeltus* sp.	Present in the insect's gut.
L. culicidarum A	*Anopheles quadrimaculatus*	
L. culicidarum C	*Culex pipiens*	
L. parva	*Calliphora* sp.	
L. muscarum	*Musca domestica*	
L. lygaeorum	*Lygaeus* sp.	Present in the latex of the plant.

The remaining two strains were isolated by Boxhall, Happold and Lloyd (1934):

Leptomonas, from *Calliphora* sp.
Leptomonas, from *Polietes lardaria*.

The following *Leishmania* strains have also been employed:

Leishmania tropica, from the National Collection of Type Cultures.
L. tropica ("Mouse")
L. tropica ("Baghdad") } From Dr Adler.
L. ceramodactyli

Leptomonas ctenocephali, from the dog flea, *Ctenocephalus canis* (National Collection of Type Cultures), has been studied along with the *Leishmania* forms, as its cultural requirements appear to be the same.

Stock cultures were subcultivated once a week, using 0·3 c.c. of inoculum and incubated for 7 days at 22° C. The *Leptomonas* forms were cultivated in bouillon, liver broth, Parke Davis peptone water, Locke serum agar and Locke serum solution[1]. It was found that, almost without exception, a good growth

[1] Liver broth contains: 0·5 g. of finely chopped, steamed rabbit's liver to 9 c.c. of bouillon. It is sterilised in the autoclave.

Locke's solution: sodium chloride, 0·9 g.; potassium chloride, 0·42 g.; calcium chloride, 0·42 g.; sodium bicarbonate, 0·02 g.; dextrose, 0·15 g.; distilled water, 100 c.c.

Locke serum solution: 4·5 c.c. Locke's solution + 0·5 c.c. filtered sheep's serum.

Locke agar: 1 litre Locke's solution + 70–80 c.c. melted, clear agar, steamed, 1 hour, *p*H adjusted to 7·4, autoclaved.

Locke serum agar: 4·5 c.c. Locke agar + 0·5 c.c. filtered sheep's serum.

Locke blood agar: 4·5 c.c. Locke agar + 0·5 c.c. sterile defibrinated rabbit's blood.

was obtained after 3 days which reached a maximum within 7 days. The growth was much greater in bouillon and liver broth, where a pellicle formed at the surface, than in Locke serum agar. Since in our hands the *Leishmania* forms and *Leptomonas ctenocephali* did not grow consistently in Locke serum agar, they were cultivated in Locke blood agar. With the exception of *Leishmania tropica* ("Baghdad"), which grew densely, these cultures were never thickly populated and only reached a maximum after 10 days.

In determining the limiting concentrations of the bactericidal substances which permit growth of the micro-organisms, all the five types of media previously described were initially used. It was found, however, that on the one hand liver broth, Parke Davis peptone water and bouillon gave almost identical results, as did also Locke serum agar and Locke serum solution on the other. In the later experiments, only bouillon and Locke serum agar were used. The *Leishmania* forms and *Leptomonas ctenocephali* were tested in Locke blood agar.

In obtaining the required solutions of the bactericidal substances in the highest concentrations employed, the compound was dissolved directly in the medium studied and lower concentrations were made from these. In every experiment suitable controls were included. With the exception of brilliant green, complete solution was assured in the highest concentration by heating in a hot water-bath for 15 min., the serum and blood, when required, being added afterwards. The anilquinolines are relatively insoluble, and in order to assess the solubility of their solutions, in the media, changes of colour and evidence of precipitation were noted.

Inoculations of the Protozoa were made using 0·05 c.c. of a 9-day culture. In all cases the inoculations were made into the various media from cultures grown in similar media; thus experiments conducted with bouillon would start from cultures in bouillon and so on. The cultures were incubated at 22° C. and examined microscopically after periods of 3, 7, 10 and occasionally 14 days. The results recorded below are taken from the 10-day readings unless otherwise stated.

Since the bactericidal action of the compounds tested was at a minimum in 48-hour bouillon cultures, and since also we desire to record these values at their lowest (the guts of the insects from which many of our flagellates have been isolated are full of bacteria), we have inoculated all our test media with a loopful of a 48-hour bouillon culture of each of the bacteria used. Growth has been tested by subculture on to agar after 1, 3, and 10 days. There are a greater number of positive cultures after 10 days, so these have been used for purposes of comparison. Cultivation was also tried in different media. Locke serum agar and bouillon are tabulated as representative so that confirmatory results are really many more than reported.

In determining the lethal concentrations of the bactericidal substances for both flagellates and bacteria the solvent employed was Locke's solution. The material used was taken from 10-day bouillon cultures of *Leptomonas* forms

and from 72-hour bouillon cultures of the bacteria. Two c.c. of the cultures were centrifuged for a quarter of an hour at high speed, the supernatant fluid was removed and the deposit resuspended in an equal volume of Locke's solution. This procedure was repeated and 1 c.c. of Locke's solution added to the deposit and well mixed. The mixture was divided equally among three tubes, to each of which was added either 1 c.c. of Locke's solution or 1 c.c. of Locke's solution containing the bactericidal substance in suitable dilution. The tubes were left at room temperature. The *Leptomonas* forms were examined, for motility, at hourly intervals for the first 6 hours and then at daily intervals, and on each occasion 0·1 c.c. inoculated into bouillon and incubated at 22° C. for 7 days, to test for viability. Bacterial suspensions were subcultivated at similar intervals by transferring a loopful into bouillon and incubating overnight at 37° C. to test for viability.

The lethal concentrations of the bactericidal substances, in the absence of nutrient media, have not yet been determined for the *Leishmania* forms or *Leptomonas ctenocephali*.

In presenting these results we have thought it best to deal with each bactericidal substance in turn. The results obtained in the presence of media are recorded in tabular form, whereas the direct bactericidal or "flagellaticidal" actions have been summarized. The figures in the tables record positive cultures.

Results

Brilliant green

With brilliant green, at the concentrations used, there was no precipitation and no colour change.

At concentrations of 1/100,000 in Locke's solution, the *Leptomonas* of *Polietes* and *Calliphora*, and *Leptomonas lygaeorum*, were non-motile after 72 hours and other strains unaffected, whilst all the *Leptomonas* forms were non-viable after 24 hours. *Staph. aureus* was non-viable after 2 hours, whereas *Bacillus coli* and *B. prodigiosus* were viable in this concentration after 72 hours.

In culture the inhibitory action of brilliant green is greater for the protozoa than for the bacteria studied. It is therefore useless for our purpose.

Table I. *Growth with brilliant green (concentration in thousands)*

	Locke serum agar				Bouillon			
	C*	1/1000	1/600	1/100	C	1/1000	1/600	1/100
Leptomonas of *Polietes*	2	.	.	.	2	2	1	1
L. parva	2	.	.	.	2	1	1	.
L. muscarum	2	.	.	.	2	1	1	.
L. oncopelti 2	2	.	.	.	2	2	.	.
L. culicidarum A	2	.	.	.	2	2	1	.
L. culicidarum C	2	.	.	.	2	1	1	.
Leptomonas of *Calliphora*	2	.	.	.	2	2	1	.
L. lygaeorum	2	.	.	.	2	.	.	.
Staph. aureus	2	2	2	2	2	2	2	2
Bacillus coli	2	2	2	2	2	2	2	2
B. prodigiosus	2	2	2	2	2	2	2	2

* C = control throughout tables.

Gentian violet

No colour changes or precipitates were observed. The flagellates lost their motility in Locke's solution after 72 hours in concentrations of 1/10,000 and after 4 hours in concentrations of 1/1000. None of the suspensions were viable. *Staph. aureus* was non-viable after 48 hours in concentrations of 1/11,000 and after 1 hour at 1/1000, but neither concentrations of the dye-stuff had any effect on *Bacillus coli* or *B. prodigiosus*. This substance is obviously of no practical importance for our present purpose.

Table II. *Growth with gentian violet (concentration in thousands)*

	Locke serum agar				Bouillon			
	C	1/11	1/6	1/1	C	1/11	1/6	1/1
Leptomonas of *Polietes*	2	1	.	.	2	.	.	.
L. parva	2	.	.	.	2	.	.	.
L. muscarum	2	2	1	.	2	2	.	.
L. oncopelti 2	2	.	.	.	2	.	.	.
L. culicidarum A	2	1	.	.	2	.	.	.
L. culicidarum C	2	2	.	.	2	1	.	.
Leptomonas of *Calliphora*	2	2	1	.	2	1	.	.
L. lygaeorum	2	1	.	.	2	1	.	.
Staph. aureus	2	.	.	.	2	.	.	.
Bacillus coli	2	2	2	2	2	2	2	2
B. prodigiosus	2	2	2	2	2	2	2	2

Results taken from 7-day readings. After 2 days *Leptomonas* of *Calliphora* and *L. lygaeorum* grew at concentrations of 1/6000 in bouillon. After 10 days *Leptomonas* forms were all dead, in presence of gentian violet. There is therefore increased action with time. The same is true with the bacterial cultures.

2 (p-*dimethylaminoanil*)-6-*acetylaminoquinoline methochloride and methosulphate* (109 *and* 115)

Three experiments gave positive cultures, with all the leptomonads used, in Locke serum agar, on all occasions, at concentrations of 1/5000. With the exception of single cultures of the *Leptomonas* from *Polietes*, *L. parva* and *L. oncopelti* 2 at 1/5000, the same was true of bouillon cultures. It was not found possible to obtain solutions of these substances at 1/1000, and indeed 1/5000 solutions in bouillon precipitated after 24 hours. *Staph. aureus* did not grow in concentrations varying between 1/5000 and 1/30,000, but *Bacillus coli* and *B. prodigiosus* have all grown in concentrations of 1/5000.

It should be noted that growth of the *Leptomonas* forms, in media containing the above substances, causes a loss of pigmentation, presumably due to reduction. It is restored on heating.

In the experiments on motility and viability in Locke's solution, all the *Leptomonas* forms were motile and viable at 1/5000 after 48 hours; but since the *Staphylococcus* alone, of the bacteria tested, was non-viable under these conditions, it seems unlikely that these substances can be used for selective cultivation of leptomonads.

Acriflavine

There was no precipitation of acriflavine in Locke serum agar at 1/1000. In bouillon a slight precipitate in concentrations in excess of 1/6000 was noticeable after 3 days, and after 7 days there was a slight precipitate at 1/11,000.

Generally speaking, all the flagellate suspensions were motile in Locke's solution containing acriflavine in concentrations of 1/11,000 after 72 hours and in concentrations of 1/1000 after 24 hours. The capacity of the flagellates to reproduce themselves from these suspensions usually followed these results; motile suspensions reproduced themselves, except in one experiment, where none of the suspensions reproduced though all were motile after 24 hours. With the bacteria, the results obtained in 1/11,000 were irregular. In concentrations of 1/1000, *Staph. aureus* was non-viable after 72 hours, *Bacillus coli* after 48

Table III. *Growth with acriflavine (concentration in thousands)*

	Locke serum agar				Bouillon			
	C	1/11	1/6	1/1	C	1/11	1/6	1/1
Leptomonas of *Polietes*	5	5	2	.	5	5	4	1
L. parva	5	4	1	.	5	4	3	.
L. muscarum	5	5	1	.	5	4	3	.
L. oncopelti 2	5	2	1	.	5	5	3	1
L. culicidarum A	5	4	2	.	5	4	4	.
L. culicidarum C	5	2	2	.	5	5	4	.
Leptomonas of *Calliphora*	5	3	2	.	5	5	4	.
L. lygaeorum	5	2	1	.	5	3	3	.
Staph. aureus	5	1	1	.	5	.	.	.
Bacillus coli	5	1	1	.	5	1	1	.
B. prodigiosus	5	5	4	3	5	5	5	.

hours, but *B. prodigiosus* was dead after 24 hours. Thus preliminary treatment of natural material containing insect flagellates with acriflavine is only likely to be of value with organisms which behave towards this substance similarly to *B. prodigiosus*.

Comparing the two sets of results, with the bacteria, we find that in the presence of media *Staph. aureus* and *Bacillus coli* are killed before *B. prodigiosus*, whilst in the absence of media the reverse is true. These results suggest that the action of this compound on *Staph. aureus* and *Bacillus coli* is bacteriostatic rather than bactericidal, inhibiting reproduction by its presence, rather than causing permanent injury to the organism's capacity for reproduction, whereas with *B. prodigiosus* the bactericidal and bacteriostatic concentrations of the acriflavine are very similar. Special experiments were devised to test this hypothesis, and it was noted that preparations of *B. prodigiosus* in media containing acriflavine at threshold concentrations, if showing a few colonies on plating after 24 hours, at 3–10 days gave viable counts indistinguishable from those obtained with normal controls, whilst similar preparations of *Staph. aureus* and *Bacillus coli* which showed definite growth after 24 hours tended to become reduced in viability with the passage of time.

The intermediate position which the *Leptomonas* forms appear to occupy,

between *Staph. aureus* and *Bacillus coli* on the one hand and *B. prodigiosus* on the other, suggests that acriflavine might occasionally be used to advantage in the technique of Boxhall, Happold and Lloyd, but since the margin between the effects of this compound on the bacteria studied and on the *Leptomonas* forms is not so great as with some other compounds tested, its use cannot be generally advocated for this purpose.

The condensed product of p-*nitrosomethyltetrahydroquinoline methochloride and* 6-*acetylaminoquinoline methochloride* (113)

This compound is relatively insoluble in concentrations of 1/5000 and above. On heating a purple colour is obtained which persists longer in Locke serum agar than in bouillon. In the latter, after 3 days the colour is absent in 1/30,000 and 1/55,000, and after 10 days it is absent in all concentrations. In Locke serum agar after 3 days there is no colour change, but after 10 days the colour is diminished in 1/5000 and absent in lower concentrations. No precipitate occurs in Locke serum agar, but in bouillon a green precipitate is formed in 1/1000 after 3 days which redissolves on heating to give the original purple coloration.

A shortage of material rendered adequate tests of the lethal action of this substance on the micro-organisms, in the absence of media, impossible.

From results obtained in media (Table IV), this substance is able to destroy bacteria in much weaker concentrations than those which allow the growth of the *Leptomonas* forms. It must therefore be included in our list of probable selective agents.

Table IV. *Growth with the condensed product of* p-*nitrosomethyltetrahydroquinoline and* 6-*acetylaminoquinoline methochloride* (*concentration in thousands*)

	Locke serum agar					Bouillon				
	C	1/55	1/30	1/5	1/1	C	1/55	1/30	1/5	1/1
All *Leptomonas* forms	5	5	5	5	5	5	5	5	5	5
Staph. aureus	5	3	.	.	.	5	3	.	.	.
Bacillus coli	5	5	3	1	.	5	4	3	.	.
B. prodigiosus	5	5	5	5	.	5	4	4	4	.

Quinanil (48s)

In solutions of this compound in media there were no marked colour changes. After 10 days there was some precipitation in bouillon but none in Locke serum agar.

In the presence of quinanil the motility and viability of the flagellates did not run parallel as they did with acriflavine. Suspensions in Locke's solution containing quinanil at concentrations of 1/11,000 and 1/1000 were all motile after 72 hours and the former were also viable. All the *Leptomonas* forms were viable in 1/1000 quinanil Locke solution after 24 hours, *Leptomonas muscarum*, *L. culicidarum* A, *L. culicidarum* C and *L. lygaeorum* after 48 hours but none

after 72 hours. *Staph. aureus* suspensions in 1/1000 quinanil Locke solution were dead after 24 hours, but *Bacillus coli* and *B. prodigiosus* were still viable after 72 hours. With 1/1000 quinanil Locke solution, *B. coli* was invariably and *B. prodigiosus* generally dead in 24 hours, though on one occasion the latter was still viable after 72 hours.

In media quinanil exerted a greater lethal action on the *Leptomonas* forms and the bacteria, tested in Locke serum agar or Locke egg agar, than in bouillon, and the least action in serum bouillon. The results with Locke egg agar and Locke serum agar were practically identical; presumably the small amount of agar present enhanced the bactericidal or "flagellaticidal" action of the quinanil. The results in Table V show a definitely greater bacteriostatic or bactericidal action for *Staph. aureus* and *Bacillus coli* than for the *Leptomonas* forms. It can readily be understood from these experiments that the preliminary treatment, in quinanil Locke solution, of naturally occurring suspensions of *Leptomonas* forms, followed by inoculation of the treated suspensions into media containing this compound in different concentrations, should form, as it did, the basis for the successful isolation and purification of cultures of *Leptomonas* forms.

Table V. *Growth with quinanil (concentration in thousands)*

	Locke serum agar						Bouillon					
	C	1/55	1/30	1/11	1/6	1/1	C	1/55	1/30	1/11	1/6	1/1
Leptomonas of *Polietes*	5	5	5	2	.	.	5	5	5	5	3	.
L. parva	5	5	5	2	.	.	5	5	5	5	3	.
L. muscarum	5	5	5	1	.	.	5	5	5	5	3	.
L. oncopelti 2	5	5	5	1	.	.	5	5	5	5	4	.
L. culicidarum A	5	5	5	2	.	.	5	5	5	5	3	.
L. culicidarum C	5	5	5	2	.	.	5	5	5	5	3	.
Leptomonas of *Calliphora*	5	5	5	1	.	.	5	5	5	5	3	.
L. lygaeorum	5	5	5	2	.	.	5	5	5	5	3	.
Staph. aureus	5	.	.	.	.	.	5	5	5	2	.	.
Bacillus coli	5	.	.	.	.	.	5	5	5	3	2	.
B. prodigiosus	5	.	.	.	.	.	5	5	5	3	3	.

2 (p-*dimethylaminoanil*)-*β*-*naphthoquinoline methochloride* (52)

This compound is relatively insoluble at concentrations of 1/5000 and above; on heating it gives a purple colour in Locke serum agar and no true colour in bouillon where, in concentrations of 1/5000 and above, the normal yellow colour is darkened to brown. The purple colour in Locke serum agar persists for 3 days but after 10 days is absent in 1/55,000 and 1/30,000. Precipitation occurs in bouillon after 10 days in concentrations of 1/5000 and above, but there is no precipitation in Locke serum agar.

Suspensions of *Staph. aureus*, *Bacillus coli*, *B. prodigiosus* and the flagellates were subjected for 72 hours to compound 52 in concentrations of 1/11,000, 1/5000 and 1/1000. In all experiments the flagellates were motile and viable for much longer periods of exposure than were the bacteria. To give one example; all flagellates were motile and viable after 72 hours at 1/11,000 and after 48 hours at 1/1000. At this latter concentration none were motile and

yet all were viable after 72 hours. All the bacteria were non-viable after an exposure of between 24 and 72 hours at 1/11,000 and under 24 hours at 1/1000.

Consideration of these results in conjunction with Table VI suggests that this compound might be very useful for the isolation of insect flagellates in pure culture.

Table VI. *Growth with* 2 (p-*dimethylaminoanil*)-*β-naphthoquinoline methochloride* (*concentration in thousands*)

	Locke serum agar					Bouillon				
	C	1/55	1/30	1/5	1/1	C	1/55	1/30	1/5	1/1
All *Leptomonas* forms	4	4	4	4	.	4	4	4	3	.
Staph. aureus	4	.	.	.	.	4	.	.	.	.
Bacillus coli	4	.	.	.	.	4	1	1	.	.
B. prodigiosus	4	1	1	.	.	4	3	3	.	.

Table VII. Leishmania *forms and* Leptomonas ctenocephali *in Locke blood agar containing the various bactericidal substances* (*concentration in thousands*)

									2 (*p*-dimethylaminoanil)-6-acetylaminoquinoline							
	Brilliant green				Gentian violet				Methochloride				Methosulphate			
	C	1/1100	1/600	1/100	C	1/11	1/6	1/1	C	1/55	1/30	1/5	C	1/55	1/30	1/5
Leishmania tropica, *L. ceramodactyli*, *L. tropica* ("Mouse")	2	.	.	.	2	.	.	.	2	.	.	.	2	.	.	.
Leptomonas ctenocephali	2	1	1	.	2	.	.	.	2	.	.	.	2	.	.	.
Leishmania tropica ("Baghdad")	2	2	2	2	2	1	.	.	2	2	2	2	2	2	2	2

Table VII (*cont.*)

									2 (*p*-dimethylaminoanil)-*β*-naphthoquinoline methochloride			
	Acriflavine				Quinanil							
	C	1/11	1/6	1/1	C	1/11	1/6	1/1	C	1/11	1/6	1/1
Leishmania tropica, *L. ceramodactyli*, *L. tropica* ("Mouse")	2	.	.	.	2	.	.	.	2	.	.	.
Leptomonas ctenocephali	2	.	.	.	2	.	.	.	2	.	.	.
Leishmania tropica ("Baghdad")	2	2	2	.	2	2	2	.	2	2	2	2

SUMMARY AND DISCUSSION

The results described above indicate that quinanil and the compound 2 (*p*-dimethylaminoanil)-*β*-naphthoquinoline methochloride might be successfully used as selective reagents for the isolation of insect flagellates of the genus *Leptomonas*.

The actual concentrations, which might be successfully used in the culture media, will show some variation according to whether the flagellate is adapted, at the time, to luxuriant growth in the media used. For example, in preliminary experiments, the *Leptomonas* from *Polietes* and *Calliphora* grew better

in Locke serum agar than in bouillon, and quinanil was more toxic in the latter than in the former. In the present experiments the reverse held, but in later ones, not here recorded, where the flagellates have again grown more abundantly in Locke serum agar, growth regularly occurs in 1/6000 concentrations of quinanil in this medium. There is little doubt from other results, not yet published, that some *Leptomonas* forms can be made to show modification of cultural requirements through adaptation. It is probable therefore that in the attempted isolation of fresh forms from the gut of the insect, several kinds of media might have to be employed.

After this work was completed, in several attempts made to purify laboratory-contaminated cultures, success was attained by following the suggested methods, using quinanil and 2 (*p*-dimethylaminoanil)-β-naphthoquinoline methochloride. The compound 113 was even less toxic to the *Leptomonas* forms in media than was the compound 52, but its bactericidal action was correspondingly weaker. The few experiments conducted in the absence of media indicated that this substance might have proved very useful, but unfortunately the material gave out and further supplies were not available. In the preliminary treatment of gut contents, acriflavine, owing to its apparently bacteriostatic action, might prove of some value for destroying organisms of the *Bacillus prodigiosus* type.

Consideration of the results obtained with gentian violet, brilliant green, and the two anilquinolines, 2 (*p*-dimethylaminoanil)-6-acetylaminoquinoline methochloride and methosulphate, suggests that these compounds are of no use as selective bactericidal substances permitting the growth of *Leptomonas* forms. The combined action of two or more of these substances has not yet been studied.

As our results indicate, little success is to be hoped for in this direction with the *Leishmania* forms, which are extremely susceptible to the toxic effects of all the substances employed. Two points of interest were obtained from these results. It might be noted that *Leptomonas ctenocephali* reacted in a similar manner to the *Leishmania* forms but that *Leishmania tropica* ("Baghdad") behaved like a *Leptomonas* form. This strain appeared similar in all respects to the other *Leishmania* forms, but it was the only one readily acclimatised to media containing no blood. Its morphology also showed some modification after a time.

We are greatly indebted to Dr St John-Brooks, Curator of the National Collection of Type Cultures, and to Prof. S. Adler of the Hebrew University, Jerusalem, for certain of the cultures of *Leishmania* and *Leptomonas* used, and also to Dr K. E. Cooper for the gift of the anilquinoline derivatives and for much helpful advice.

One of us (F. C. H.) has also been in receipt of grants from the Medical Research Council, and he would like to take this opportunity of expressing his thanks to that body.

REFERENCES

ARMITAGE, G., GORDON, J., COHEN, J. B., ELLINGWORTH, S. and DOBSON, J. (1929). The use of a new quinoline derivative in the treatment of infection. *Lancet*, ii, 968.

BOXHALL, G. N., HAPPOLD, F. C. and LLOYD, LL. (1934). Quinanil as a bactericidal agent in the isolation of an insect flagellate. *Parasitology*, **26, 44.**

BROWNING, C. H., COHEN, J. B., ELLINGWORTH, S. and GULBRANSEN, R. (1926). Antiseptic properties of amino derivatives of styryl and anil quinolines. *Proc. roy. Soc.* B, **100**, 293.

—— —— —— —— (1928). Antiseptic compounds: Some further derivatives of anilquinoline. I. *Ibid.* **103**, 404.

WENYON, C. M. (1926). *Protozoology*, **1**, 318.

(*MS. received for publication* 13. II. 1935.—Ed.)

METHOD OF CHAIN FORMATION IN *HERPETOMONAS BANCROFTI*

By FRANCIS O. HOLMES

From the Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey

(With 9 Figures in the Text)

In 1928 Bancroft reported the presence of a flagellate in latex of *Ficus* in Australia. Several years later, the writer (Holmes, 1931) described this flagellate under the name of *Herpetomonas bancrofti*. The species was characterised by formation of linear chains of three to more than twenty aflagellate cells. Alternation of cell ends was always regular within these chains. Chain formation in haemoflagellates had not been reported previously, and at the time all attempts to find stages of division that would throw light on the manner in which chains were formed proved unsuccessful.

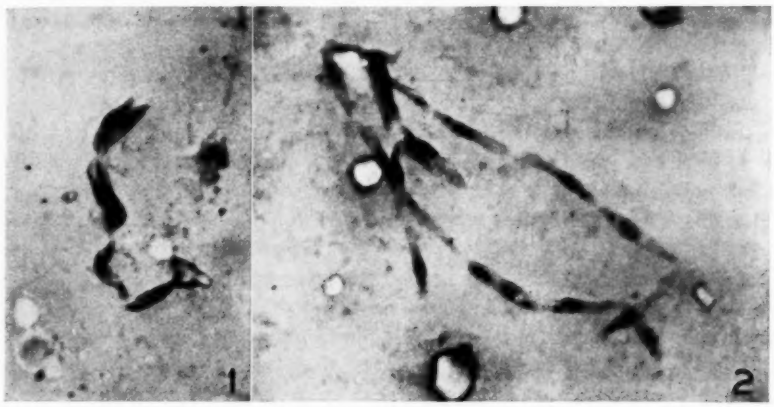
Several possible explanations of the origin of chains were considered. The most plausible of these suggested that, upon division of a single flagellate, the two new cells might remain attached by their posterior ends, and, upon maturing, might be divided into four by successive longitudinal fissions. It was supposed that division of a joined pair would begin normally at the anterior end of one individual, and that the movements of the newly formed cells might induce division of the second individual of the original pair to begin posteriorly. If some or all of the cells should remain attached after division, and if the process were repeated, long chains might arise.

Soon after *Herpetomonas bancrofti* was described, Franchini (1931, 1931*a*) reported similar chain formation in a second latex flagellate, *H. ganorae* Franchini, from *Ficus hochstetteri*. He suggested that migration of new parabasal bodies and nuclei to opposite ends of cells, followed by transverse fission, would account for lengthening of chains. Unfortunately he observed only stages of formation of pairs from isolated individuals in fixed material; it seems possible that such pairs may have been formed by longitudinal fission. No cells in chains were recorded as dividing, individually, by transverse fission, with consequent disturbance of regular alternation of cell ends. Neither had any evidence suggesting transverse fission been observed in *Herpetomonas bancrofti*, although the possibility that this type of division might occur had not been excluded.

Among a number of microscopic preparations of *H. bancrofti* in fig latex, there was one in which binucleate and anucleate flagellates, ordinarily rare, were comparatively numerous. Intensive study of this preparation has

recently yielded a small amount of direct evidence, as well as considerable circumstantial evidence supporting the earlier hypothesis of longitudinal division of the chain as a whole.

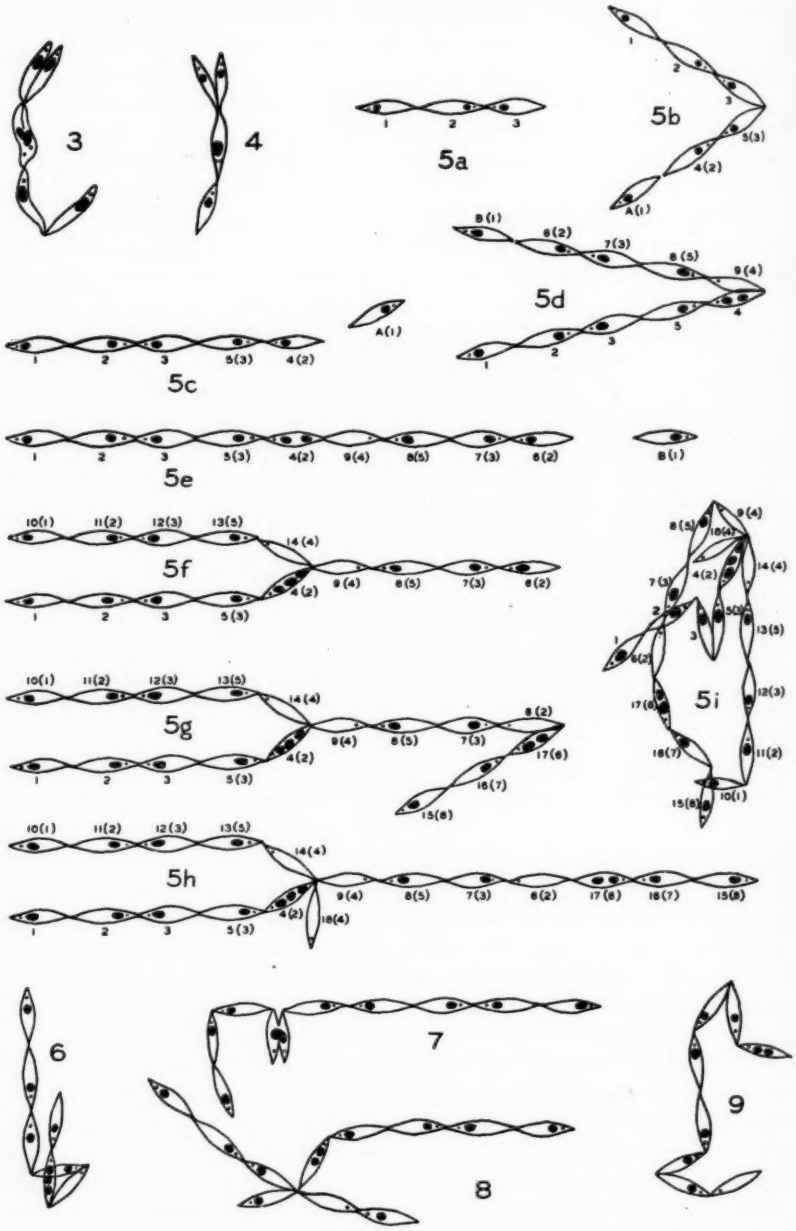
Direct evidence was furnished by observation of two short chains, that had been fixed during what appears to have been normal division. Fig. 1 (corresponding to diagram in Fig. 3), represents a chain of four cells which was in process of division to form a chain of eight. A cell at one end apparently had completed cytoplasmic division. The next had formed two parabasal bodies and two nuclei, and had begun cytoplasmic division from its posterior end. Its anterior end was attached to one of the two remaining individuals, neither of



Figs. 1–2. *Herpetomonas bancrofti*, chains of aflagellate cells. Fig. 1. Chain of four, in process of division by longitudinal fission. Fig. 2. Chain of eighteen, including one with three, one with two, and three with no nuclei. Magnification 1600 diameters.

which had begun division. Fig. 4 represents a shorter chain, in which the terminal cell that had possessed a free anterior end had completed its division; the middle cell had formed two parabasal bodies, but its nucleus was still undivided, although large; the third cell, which showed only one parabasal body and one nucleus, had not initiated division. Both of these chains displayed the long sought *Y*-form, indicating that division had not been completed at the time of fixation.

Contributory evidence was supplied by observation of a number of abnormal chains, several of which are represented diagrammatically in Figs. 5–9. Perhaps the best of this additional evidence for division of the chain as a whole was found in an aggregate of eighteen individuals, shown in the photograph reproduced in Fig. 2, and diagrammatically in Fig. 5*i*. In this aggregate there is displayed again the *Y*-form, representing in this case a division that could not be completed because of the presence of an anucleate individual within the dividing chain.



Figs. 3–9. Diagrammatic representation of chains of *Herpetomonas bancrofti*, described in text.

The original form of this aggregate (Figs. 2 and 5*i*) is so complicated that it is difficult to recognise relationships between various cells. A diagram of this same chain in extended form (Fig. 5*h*) discloses evidence supporting the theory of chain division by longitudinal fission in successive cells. In order to appreciate the significance of this evidence it is necessary to consider the probable sequence of divisions preceding the time of fixation of the preparation. It is possible to understand how this chain of eighteen cells might have been derived from three uninucleate cells broken off from a longer chain. A series of divisions has been deduced by working backward from the chain as found in the stained latex smear. This series is presented in the order of supposed occurrence. In Fig. 5*a–i*, individuals are numbered in the order of their formation, numbers in parentheses indicating cells from which these individuals immediately arose.

The first division of the original short chain (Fig. 5*a*) would naturally start at the anterior end of cell 1, extending through 2, and part way through 3. This would have formed successively cells 1 and *A* (later lost), 2 and 4, and 3 and 5 (Fig. 5*b*). The point of origin of this first division may be safely assumed, because the other end of the chain of three individuals is a posterior end of a cell, an improbable place for the beginning of division in a haemoflagellate. Cell *A* then became detached (Fig. 5*c*). There is evidence that the next division started also at the anterior end of cell 1, forming successively 1 and *B* (later lost), 2 and 6, 3 and 7, 5 and 8, and 4 and 9 (Fig. 5*d*). The pulling of the series *B*, 6, 7, 8 away from the series 1, 2, 3, 5 may possibly have hastened cytoplasmic division in cell 4, and so caused a split before the new nuclei could be properly allocated, or perhaps even before nuclear division. At any rate, cell 9 failed to receive its nucleus, which remained in 4. It is this occurrence of adjacent abnormal cells that gives evidence of the form of this second division. Cell *B* became detached (Fig. 5*e*). The third division started at the anterior end of cell 1, as both preceding divisions had done. Cells 1 and 10, 2 and 11, 3 and 12, 5 and 13, and 4 and 14 were formed, the last receiving no nucleus. The nucleus which should have gone to 14 remained as an extra nucleus in 4, making cell 4 trinucleate (Fig. 5*f*). This split stopped at anucleate cell 9, which could not divide. The inability of the anucleate cell to divide is presumably responsible for the retention of a *Y*-form in this aggregate, which in turn gives evidence for the character of this third division. The other free end of the chain was the posterior end of a cell, an unfavourable place for a division to begin. A fourth split started at the anterior end of cell 8. This is made evident by the positions of four uninucleate individuals, 8 and 15, 7 and 16, a binucleate 17, and an anucleate cell 6 (Fig. 5*g*). Trinucleate cell 4 again divided, but formed only an anucleate cell 18 (Fig. 5*h*), as individuals with more than one nucleus often do; perhaps even in this case there may have been other anucleate cells formed and lost. In the process of making the microscopic preparation, the chain was somewhat twisted, as shown in Fig. 2, and diagrammatically in Fig. 5*i*.

This large aggregate, with its *Y*-shape and both binucleate and trinucleate cells, with corresponding anucleate individuals within the chain, gives strong

support for the contention that successive longitudinal divisions of constituent cells may account for at least part, perhaps all, of such chain formation as has been observed.

Additional circumstantial evidence for this type of division of whole chains is represented by aggregates of the types shown in Figs. 6–9, each of which shows at least one binucleate or trinucleate cell and one anucleate cell so located as to suggest that their formation was incidental to this same type of chain splitting.

No evidence has been adduced to prove that transverse fission does not play a part in the formation of chains in *Herpetomonas bancrofti*. The failure to find any cell that had divided by such a process before its neighbours in a chain, with consequent disturbance of the regular alternation of cell ends, seems significant, however. Since evidence has been brought to show that successive longitudinal divisions may, and do, occur in individuals within chains, there seems to be no present need of the transverse-division hypothesis to account for occurrence of observed aggregates.

SUMMARY

Stages of incomplete division of chains of individuals in the species *Herpetomonas bancrofti*, as well as the distribution of abnormal cells within long chains, were found to support the writer's earlier view (Holmes, 1931) that successive longitudinal divisions of individuals occur within chains. In this type of division there is alternate splitting of individuals from anterior and from posterior ends of cells. It is concluded that this hitherto unproved type of division of pairs and chains is sufficient to account for the presence of all observed aggregates.

No evidence was found suggesting occurrence of any essentially different type of cell division, such as the transverse fission reported by Franchini (1931, 1931*a*) as possibly accounting for formation of chains in *H. ganorae*.

REFERENCES

BANCROFT, T. L. (1928). Flagellates in certain Queensland plants. *Proc. roy. Soc. Qd* (for 1927), **39**, 22.

FRANCHINI, G. (1931). Su di un flagellato speciale del lattice del Ficus Hochstetteri (Mig.) A. Rich. *Arch. ital. Sci. med. colon.* **12** (11), 637.

—— (1931*a*). Étude sur un flagellé spécial du latex d'un figuier de l'Érythrée (Ficus hochstetteri (Mg.) A. Rich). *Bull. Soc. Path. exot.* **24**, 843. (Also in *Deuxième Congrès International de Pathologie Comparée*, **2** (Comptes Rendus et Communications), 496. 1931.)

HOLMES, F. O. (1931). *Herpetomonas bancrofti* n.sp. from the latex of a *Ficus* in Queensland. *Contr. Boyce Thompson Inst.*, **3**, 375.

(*MS. received for publication* 15. II. 1935.—Ed.)

THE LIFE HISTORY OF *MEROCYSTIS KATHAE* IN THE WHELK, *BUCCINUM UNDATUM*

By RUTH PATTEN, Ph.D.

Department of Zoology, Trinity College, Dublin

(With Plates XI–XIV, containing Figs. 1–56, and Figs. I–X in the Text[1])

CONTENTS

		PAGE
I.	Introduction	399
II.	Material and methods	400
III.	Periodicity	401
IV.	Life history	404
	(i) Development of the young parasite	404
	(ii) Development of the female parasite and macrogamete	407
	(iii) Development of the male parasite	409
	(iv) Microgametogenesis	412
	(v) Fertilisation and first division of zygote	416
	(vi) Sporogony	419
V.	Nuclear divisions in *Merocystis*	424
VI.	Summary	426
	References	428
	Description of Plates	429

I. Introduction

In 1911 Dakin discovered a parasite which he called *Merocystis kathae* in the renal organ of the whelk, *Buccinum undatum*. Since then only one additional study has been made of this species—that by Foulon, published in 1919, though all his observations had been made before 1914.

Dakin discovered this parasite in whelks from Port Erin (I.O.M.) and classified it as a Polysporocystid Coccidian, but in his description of the life history he appears to have confused many of the stages. According to him, microgametes form at the periphery of the parasite, and the zygote divides into secondary cysts which then give rise to a mass of monozoic spores.

Foulon, who also obtained his whelks from Port Erin, showed that the male parasite divides into a number of cytomeres (Dakin's secondary cysts), each of which finally produces many microgametes. After fertilisation, the zygote nucleus divides many times, the resultant nuclei at first lying at the periphery (Dakin's microgametes) and only later sinking into the interior where the cytoplasm cleaves round them to form uninucleate sporoblasts, which subsequently become spores, each containing two sporozoites (not one, as Dakin stated).

[1] Figures occurring on Plates bear Arabic numbers, whilst text-figures bear Roman numbers.

Foulon also described the nuclear structure of the male and female parasites, and remarked on the similarity of the nucleus of *Merocystis* to that of *Aggregata*, particularly as regards the karyosome which in both these genera is large and complex. He considered that *Merocystis* certainly belongs to the family Aggregatidae, and that probably the name should be changed to *Aggregata* whenever a complete revision of the Aggregatidae should be made. No schizogony was seen by either of these observers, but they both considered it probable that one occurs in a different host. For the latest review on the classification of the Eimeriidea see Hoare, 1933.

Since 1919 nobody has recorded any observations on this parasite, although our knowledge of the Coccidia in general, and the closely related genus *Aggregata* in particular, has greatly increased. Dobell's memoir on *A. eberthi* appeared in 1925; as well as dealing conclusively with every part of the life history, it amplified his previous account with Jameson (1915) as regards the chromosome number and the reduction division of the zygote nucleus. Naville, some months later in 1925, published an account which was largely in agreement with the findings of Dobell (cf. also Bělař, 1926*a*).

The chromosome cycle in Coccidia and gregarines is very interesting (cf. Naville, 1931). In almost all species, when exact observations have been made within recent years, reduction is zygotic and not gametic. It thus seemed desirable to study the behaviour of the chromosomes in *Merocystis*, and at the same time to see whether Foulon's description could be confirmed (as indeed has largely been done). I hoped to discover the schizogony as well, but so far have not succeeded. The whelk is omnivorous, and the task is not simple, but in view of the similarity of the life histories of *Merocystis* and *Aggregata*, so far as they are known, it is possible, that the missing phases occur in a crab or other crustacean (cf. Setna and Bhatia, 1934).

At one time I thought that a protozoan parasite which I found in the foot of the whelk represented the schizogonic cycle, but this has not proved to be correct. I hope shortly to publish some notes on this previously unrecorded organism.

II. Material and methods

The whelks used for this investigation were mostly obtained from Coliemore Harbour, Dalkey, co. Dublin. They were caught by the fishermen in pots off the Muglins Island in the south of Dublin Bay; but supplies were also obtained from Port Erin in April 1933, from Plymouth, December 1933, and from Howth, co. Dublin, also in December 1933. In the three latter cases the whelks were sent to the laboratory where they were kept in sea water, constantly aerated, while examination was proceeding. This was also done for supplies from Dalkey required for intravital observation or for making smear preparations. Such of these as were subsequently fixed often gave unsatisfactory results (particularly Plymouth and Port Erin supplies). It appears, as previous workers on the Coccidia have said, to be absolutely essential that material should be fresh.

It may be added that much material, fixed under what appeared to be correct conditions, and at the same time as other specimens which gave excellent results, was entirely useless, and had to be discarded.

As regards material from Dalkey, the usual procedure was to take bottles, containing the fixative required, down to the harbour, as soon as possible after the whelks had been caught, and where meantime they were kept submerged in a pot. As the later stages of parasites are large (up to 180 μ) infected specimens can be detected by naked-eye examination, and generally only such as were seen to contain parasites were placed in fixative. Of course this is by no means an accurate method of determining the number of whelks parasitised, and many that were but slightly infected escaped detection.

Various fixatives were used, including Bouin's fluid, sublimate acetic acid (2·5–5 per cent. glacial acetic acid), Duboscq and Brasil's modification of Bouin's fluid, Dobell's sublimate formol, Schaudinn's sublimate alcohol, and Carnoy's mixture. The first three were most satisfactory. Many special fixatives were also employed in the investigation of the cytoplasmic inclusions, but these will be described in a later paper dealing specifically with this subject. Of staining methods, only three were much used. These were Heidenhain's iron-alum haematoxylin, with or without a counter-stain, and Giemsa's stain (the method followed is described in the 6th edition of the *Microtomist's Vademecum*, p. 503)—both of which gave excellent results; and, after sublimate acetic and Schaudinn's fixative, Feulgen's nucleal reagent, counter-stained with alcoholic solution of light green. This gives an exceptionally good picture of the nuclear structure of the young parasites and of males and females, and also of division figures, and was invaluable in deciphering many of the stages. The standard times proved best, *i.e.* 4 min. in hot (60° C.) hydrochloric acid, and 2 or 3 hours in fuchsin sulphurous acid (see Robertson, 1927, for details).

III. PERIODICITY

The most interesting phenomenon observed in this investigation was a periodicity in development. Material was fixed at various intervals from April 1933 to October 1934, and I found that the parasite takes almost one year to complete its full cycle. As far as I can ascertain, no such periodic development has previously been observed in Coccidia or gregarines. Dehorne (1930 *a* and *b*), however, showed that the life history of a coccidial parasite which he regards as an *Aggregata*, but which is probably a *Caryotropha* (Thomas, 1930), is correlated with the development of the host—an annelid worm. In the gregarine *Gonospora*, Hentschel (1926, 1930) found a very close correlation of the life history with the sexual cycle of the host—*Gonospora arenicolae* with its host *Arenicola ecaudata* and *Gonospora varia* with *Audouinia tentaculata*. The formation of the sporocysts is simultaneous with the attainment of sexual maturity by the worm, and the ejection of the sporocysts with the gametes of the host was observed. It was suggested that a secretion produced by the gonads stimulated the development of the parasite. It is probable that the

cycle described by Kunze (1907) in the coccidium *Orcheobius herpobdellae* from the testes of the leech, *Herpobdella atomaria*, would allow of a similar correlation with the sexual cycle of the host. It is also possible that the seasonal variation described by Duboscq in *Selysina perforans* (1918) is of the same kind as that shown by Dehorne and Hentschel. Nothing similar to the behaviour of *Merocystis* has been described, however, for the life cycle of the parasite seems to be correlated with seasonal variations, and not with any changes occurring in the organ of the host parasitised. A few authors definitely state that they have looked for, but not found, such a periodic development (*i.e.* Debaisieux in *Pseudoklossia chitonis* and *P. patellae*; Vincent in *Légerella hydropori*; and Wedekind in *Barrouxia schneideri*).

A rapid survey shows that the smallest parasites may appear in the renal organ any time from March to June. In July and August there is a gradual increase in size, but no sexual differentiation is seen until September, when early stages of microgametogenesis are also present. In October a few ripe macro- and microgametes were seen, but zygotic stages ("spindle nuclei") and early sporogonial phases only make their appearance in November. By December, as well as the latter stages, an occasional cyst is found in which sporoblastic division has occurred, though spores containing sporozoites were not seen till January. Spore stages were comparatively rare in January, but became increasingly common till by late May they were more numerous than any other stage (unless of course the whelk had been recently reinfected with merozoites, when very small parasites would also be found in abundance). There is a sudden falling off in the number of spore stages after May; in late June and July structures are often seen which are almost certainly degenerating cysts or isolated spores.

Very full notes were made on this seasonal development, but for the sake of brevity these will be greatly condensed. I do not wish to suggest that an exact parallel will be found every year, and in all localities, with the cycle which I have found for one year in whelks from one place. However, material obtained from whelks from Port Erin or Plymouth approximated very closely, as regards its developmental condition, to that obtained at Dalkey at the same date, and there is no disparity between the cycles observed in 1933 and 1934. I hope to continue the observations during the coming year, and so trace the cyclical development through two complete years.

All material, except where specific mention is made, was obtained from Dalkey and fixed at the harbour.

April 1933. Port Erin: about 33 per cent. infected. Sporogonic stages common, but all others, except very small parasites, found.

May 2nd. About 40 per cent. infected; late sporogonic stages very numerous.

May 18th. Thirty-six examined; ten infected, some very heavily. This supply provided much excellent material, and many of the drawings were made from these preparations. Some idea of the state of development can be

obtained by counting all the parasites in a single section. In one such count there were thirty-eight in late sporogony, two in early sporogony, ten undergoing microgametogenesis, and about eighteen younger parasites including males, females, and undifferentiated but already large organisms. All renal organs fixed at the same date do not show precisely the same state of development; some of the blocks of this series reveal a more juvenile condition, and though late sporogony phases are found in all, they do not preponderate. In one block, at any rate, there was evidence of a fresh infection, as many very small undifferentiated forms were found without others of an intermediate size linking them with the full-sized parasites also present.

June 9th. Twenty-six examined; four fixed, of which only one was certainly infected. A few normal full-grown parasites of all stages were present, with many degenerating cysts, some of which had been attacked by phagocytes. Sometimes cysts contained normal and degenerating spores side by side.

June 16th. Forty-nine examined; ten fixed. Some blocks contained degenerating cysts, as on June 9th; but very young parasites were abundant. They are noticeably smaller than the majority found in the next series.

July 13th. Sixty-five examined; fifteen fixed. Some also brought to laboratory and examined fresh. Smears made. This series showed an abundance of very young stages, uniform in size and structure. Although most parasites were between 30 and 60 μ in diameter, very small ones were also seen. Older stages were present, but frequently abnormal—the commonest abnormality being incomplete segmentation of the cytoplasm with abnormally large nuclei. No parasites of intermediate size were seen; so I conclude that the large forms were remnants of an earlier infection.

July 26th. Thirty-five examined; seven infected. A few also examined in the laboratory. This series is similar to the preceding, but there appears to be a slight increase in the average size of the young parasites.

September 28th. Forty-six examined; thirty-nine infected (twenty-one very heavily). An enormous advance was seen in the development as compared with the previous series. Large parasites (80 μ or more), still contained within their host cells, predominated. I also saw many free parasites, including sexual forms and the earlier stages of microgametogenesis, but no ripe microgametes; and stages after cytomeric division were comparatively rare. No very small parasites were found.

November 21st. Twenty-six examined; thirteen infected. Many were also examined in the laboratory. Ripe microgametes were present—the first time since May—and were abundant. Often the pellicle around the parasite had partly disappeared, and some of the gametes had escaped through the rupture. Mature macrogametes and "spindle nuclei" stages were present, but no late sporogonic stages.

December 11th. Thirty-one whelks from Howth examined in the laboratory; nine infected. Fixation was poor, but the developmental state of the parasites was not significantly different from the previous Dalkey series.

December 14*th*. Twenty-five whelks from Plymouth examined; thirteen infected (about eight would have been detected superficially). Similar to Howth series.

December 15*th*. Twelve whelks from Dalkey examined in the laboratory; eleven infected (only about six would have been noticed at the harbour). Good fixation. Considerable advance in development over the last Dalkey series was perceptible. Parasites contained within host cells were not numerous, and all showed sexual differentiation. Late sporogonic stages were also found—for the first time since June—but no mature sporozoites.

January 30*th*, 1934. Twenty-five examined; thirteen infected. As for December series, except that a few cysts containing mature spores were present and sporoblastic stages were much commoner. Owing to storms which broke the pots, the fishermen were unable to supply me with any more whelks until

March 31*st*. Forty examined; eleven infected, but mostly not very heavily. This series did not show the same uniformity as the others. Minute forms indicating a fresh infection were found in some of the blocks. In all, however, some spore stages were present, and the series corresponds quite well with material obtained from Port Erin almost one year before.

May 31*st*. Fifty-three examined; about eighteen infected. Agrees well with May 18th, 1933. A few degenerating cysts seen occasionally.

July 13*th*. Fifteen examined; six fixed, but infection noticeable macroscopically in only one. Agrees with observations on same date in previous year.

July 28*th*. Only young forms seen.

August 4*th*. Nine fixed, no infection macroscopically. A few degenerating late stages and young forms showing increase in average size.

August 24*th*. Nine fixed. Further increase in average size. Sexual differentiation apparent in some, but no ripe gametes.

October 19*th*. Forty-eight examined, infection apparent in most; nine fixed. Considerable advance in development as compared with September 1933; a few very early sporogonic stages.

IV. Life history

(i) *Development of the young parasite*

The smallest parasites found were about 10μ in diameter and were already intracellular. Each parasite was presumably derived from a merozoite which had found its way into the renal organ and penetrated an epithelial cell. As growth takes place the surrounding host cell hypertrophies, and its nucleus becomes a complicated branching structure occupying most of the cell, while such cytoplasm as is present becomes greatly vacuolated (Fig. I). In sections this nucleus appears lobulated, and one might think that many nuclei surround a single parasite; but smear preparations make it clear that the parasite is contained within a single cell, whose nucleus hypertrophies but does not really divide. Usually the host cell retains its position in the renal epithelium,

though with increase in size it projects into the cavity of the organ or the underlying connective tissue. There is no evidence that the parasites ever penetrate cells of this tissue, but they retain their intra-epithelial position throughout the growth period. Figs. 1–4 show young parasites; in these the nucleus is relatively very large, and possesses a centrally placed karyosome of considerable size. The nucleus is lightly stained except near the surface, where there is a ring of denser achromatinic substance containing rods and granules which stain intensely with basic dyes. With Feulgen's method (Figs. 2, 3, 4) this border of the nucleus is a deeper green (achromatinic) than either the central part of the nucleus or the surrounding cytoplasm, while the rods and granules give the chromatin (nucleic acid) reaction of deep mauve. The karyosome is green, but either within, or plastered on to it, one sees fairly large granules (or a single granule) of intense mauve colour; evidently the portions plastered on to the karyosome are in process of entering it. Foulon

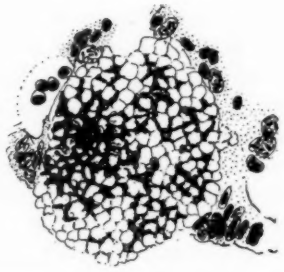


Fig. I. Specimen fixed September. Bouin, Giemsa's stain. ×420. Host cell and nucleus.

does not show such a structure in the smallest forms, but this can probably be accounted for in that the stain he used was iron haematoxylin which often renders the whole karyosome uniformly dark. He does, however, figure an intrakaryosomic body in larger organisms, and says, referring to *Aggregata*: "Léger et Duboscq admettent la pénétration d'un caryosome sidérophile dans le nucléole de plastine. Il nous paraît fort peu probable que cette description se vérifie dans l'espèce qui nous intéresse." Dakin also shows a compound structure of the karyosome in larger parasites. In *Aggregata eberthi*, Dobell has shown a single chromatinic body, actively penetrating into the karyosome through a micropyle. He refers to this body as the micronucleus, and it appears to be the repository of the chromatin during the growth period, while all the material for the chromosomes of the subsequent division figures appears to be derived from it. A similar nuclear structure has been described by Jameson (1920) in the gregarine *Diplocystis schneideri*. In the intracellular phases of *Merocystis* one or more bodies are found within the karyosome. These stain like chromatin, and persist throughout the whole growth period; and in mature forms, both male and female, assist in the formation of the network from which

the chromosomes are later derived. Such bodies differ, however, from those seen in *Aggregata*, first in being less regular in shape, and secondly in that they do not possess all the chromatin. In *Merocystis*, granules of chromatin are also left outside the karyosome in the ring of darkly stained achromatinic substance; and though the mauve coloration (with Feulgen's reagent) in this region becomes less and less conspicuous as growth proceeds, there is no evidence that this is due to migration of the chromatin into the karyosome. Chromatin granules (mauve) seem to reappear *in situ* in the maturing males and females, and provide some of the material for the network. I shall therefore refer to the granule, or complex of granules, within the karyosome as the intrakaryosomic body.

The nucleus, as mentioned above, is at first relatively large, but during the growth period there is a change in the ratio of cytoplasm to nucleus, the former increasing relatively more rapidly. The ring (really a hollow sphere) of dense nucleoplasm containing the chromatin granules at first lies close to the periphery of the nucleus (Figs. 1, 2, 3), but later as the nucleus increases in volume it is found some distance from the surface as in Fig. 5. It then becomes thin at certain parts, and only partially encircles the karyosome (Fig. 6); further disintegration occurs, and the fragments pass once more to the periphery of the nucleus where they are seen as irregular threads and blebs of more deeply stained achromatinic material (Fig. 7). The mauve-staining granules have by this time almost disappeared (Fig. 7), though in some Feulgen preparations there is in this part of the nucleus a very faint mauve coloration.

The karyosome has meantime grown considerably and it can now be seen that there is a segregation into an outer and inner region, the outer part being more alveolar, though there is no very clear line of demarcation. The intrakaryosomic structures are usually in the form of hollow spheres, as is seen in Fig. 7. The karyosome in full-grown but sexually undifferentiated forms is characteristically spherical or subspherical, with an outer fine alveolar layer, and contains the usual chromatin complex, while no chromatin granules can be seen outside the karyosome. Haematoxylin staining frequently renders these stages so dark that it is difficult, though not usually impossible, to perceive the true structure. It seems singular that there is no differentiation into males and females until growth is almost complete. From Foulon's description it would appear that he too noted this, though he figures quite small forms as "macrogametocytes". The similarity of young parasites, and also of all the well-grown but intracellular forms seen in some preparations, is strong evidence that sexual differentiation does not occur until after this time. In many Coccidia sexual differentiation is shown by the staining reaction of the cytoplasm, but in *Merocystis* all the intracellular parasites are alike. Their cytoplasm is lightly coloured and contains coarse alveoli; but when once they are free of the host cells, it stains more deeply and has smaller alveoli. This change occurs *after* sexual differentiation, in both males and females. The figures unfortunately give no good idea of this sudden alteration in staining power, which is

particularly noticeable in slides stained by Giemsa's method. In these even low-power inspection at once reveals the difference between young intracellular phases and the later ones after the parasite is freed.

(ii) *Development of the female parasite and macrogamete*

Fig. 8 shows the nucleus and part of the cytoplasm of a parasite which can definitely be distinguished as a female. The nucleus is lightly stained and shows no chromatin granules outside the karyosome, which is spherical and alveolated, and clearly differentiated into an outer and more lightly stained zone and an inner denser portion. The intrakaryosomic body is present as a hollow spherule, and three small granules. The parasite is intracellular and 165 μ in diameter. Such stages were particularly common in material fixed in November, December, and January. They are mature female parasites, and except possibly for some further growth no changes occur until each begins to metamorphose into the macrogamete. The first step in this process is apparently the extrusion of the intrakaryosomic bodies; this has not been observed, but it seems a safe inference judging by such forms as Figs. 9, 10, 11. Fig. 9 shows the nucleus of a female with a spherical karyosome; the latter is large (15 μ) but not so large as that seen in Fig. 8 (20 μ). It is homogeneous in structure, except for some alveoli. Close to it is a dumbbell-shaped and deeply stained chromatin body (*B*), attached to a small sphere of achromatinic substance (*A*), the composite structure being very similar to the intrakaryosomic body from which I am sure it is derived. Some not very deeply stained (mauve) granules of chromatin are seen near the periphery of the nucleus, and are probably due to a recondensation of the granules of chromatin which disappeared in the earlier stages. At this time too occurs the change in the cytoplasmic staining and the freeing of the organism from its enveloping host cell which is apparently due to contraction of the parasite. The two processes go hand in hand and I have never seen a deeply stained parasite still surrounded by its host cell, or a lightly stained one free.

A later stage in macrogametogenesis is shown in Fig. 10. The chromatin granules which are very fine and numerous are forming a kind of network, and are situated in material more intensely stained with the light green than is the rest of the nucleoplasm. There are as well three larger rings or blebs of deep mauve-dyed chromatin (*B*) derived from the intrakaryosomic bodies, and bound to two of these are small green-stained spheres (*A*) similar to the structure in the previous figure and presumably extruded from the karyosome with the chromatinic blebs. Much evidence for the latter phenomenon is derived from the structure of the karyosome in this figure, which has a large clear vesicle at one point, very close to one of these bodies. Fig. 11 shows further development of the chromatin to form threads surrounding the centrally placed karyosomic remnant. The drawing should show chromatin threads above and below the karyosome, but these were omitted, as it would have looked as though they were within that body. At *A* is an achromatinic

spherule with chromatin bleb attached, corresponding to the structures similarly marked in Figs. 9 and 10. By this time the nucleus has usually come to lie close to the surface.

The next stage (Fig. 12) shows that the chromatin threads have collected to form a complex network. This figure is a reconstruction (as indeed are most of the others), but great care was taken to make it as accurate as possible, though the central part was so entangled that the task was there impossible. The threads, as will be noticed, are very fine and have not the clear outline observable in later stages. The nucleus (including that part which contains the karyosome and some of the chromatin) abuts on the surface.

This stage is followed by one which may be called the "pre-fertilisation cobweb" stage (Fig. 13), in which the threads are clearly defined and very deeply stained, and sometimes bear enlargements. Another feature in the "cobweb" stage is that the threads or loops nearly all lie close, or are attached, to the periphery of the nucleus throughout their entire length, and not merely at the extremities. The central mass shown in Fig. 13 is not formed of an unorganised clump of chromatin, but of coiled loops. A slight contraction and thickening of the threads of the cobweb occurs. All these network stages (cf. Figs. 12 and 13) agree in almost always retaining a fairly large karyosome. They were often seen in preparations made in November, December, and January. Further contraction of the cobweb network proceeds, till a compact and much coiled one such as seen in Fig. 14 is found. This figure is drawn from a mature macrogamete. The fine point of chromatin lying in the drawing just below the karyosome was in reality on the very surface of the nucleus, and only 2 or 3 μ from the periphery of the parasite; while the nucleus itself was somewhat elongated, with its long axis almost at right angles to that of the parasite itself. Unfortunately no specimens were seen cut longitudinally through such a nucleus; but very careful note was made of the parasites seen at this stage, and in all the chromatin was very close to the nuclear surface. The pellicle is fairly thick, but is not to be confused with the much thicker membrane formed after fertilisation.

Foulon's investigation of the female parasite and gamete will be discussed at the end of the next section, but he did not trace the metamorphosis of the macrogamete. I have not seen individual chromosomes at any stage in the ripening of the macrogamete of *Merocystis*, as has been described in *Aggregata* (see Dobell, 1925). A considerable quantity of material was collected at times of the year when the maturing of gametes was taking place, and stages such as shown in Figs. 12–14 were not uncommon. Nevertheless, I hope to collect more material this season and to examine this question more carefully.

Naville (1925, 1931) has described the formation of "secondary nuclei" in the development of the macrogamete of many Coccidia including *Aggregata*. He regards such nuclei as representing a degenerate form of the divisions which he recounts in some other genera; and such divisions, producing a limited number of gametocytes from a single merozoite, he calls "gamogony".

I have found no bodies at any time in *Merocystis* comparable to these "secondary nuclei" nor anything like "gamogony".

(iii) *Development of the male parasite*

Differentiation into a male may occur while the parasite is still very small, particularly where parasites are packed closely together in the renal organ, when even such comparatively mature nuclear conditions as seen in Figs. 18 and 19 may be found in organisms only 50–70 μ in size. The average size of adult males appears to be somewhat less than that found in females, but no satisfactory figures can be given. A vast number of such measurements would have to be made, and as there is obviously much variation it did not seem

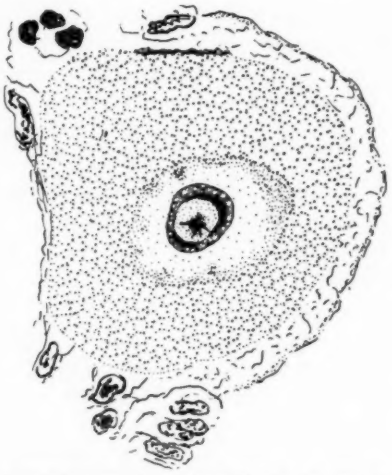


Fig. II. September. Bouin, stained Giemsa. Section 5 μ. ×625.

advisable to devote time to such a task. (Measurements were made of late male gametogenesis and late sporogony stages, derived from male and female parasites respectively, and there, though it was quite obvious that the former were usually smaller than the latter, there was much overlapping.)

Fig. II shows a parasite, with diameter of 80 μ, which can already be regarded as a male, though the organism may reach a much greater size before the sex can be determined. The distinguishing marks are found in the structure of the karyosome, which is often oval and has a darkly stained and alveolated rim with a paler internal portion, the whole containing the usual intrakaryosomic complex. The nucleus outside the karyosome shows no chromatin at all, but the nucleoplasm is denser towards the periphery. The karyosome increases enormously in size in older males, the narrow marginal region alone staining darkly, the central part being very pale except for a mass surrounding the blebs of chromatin, and sometimes a few strands passing from this to the rim.

The whole karyosome loses its spherical form and becomes oval in section (Fig. 15). The outer part of the nucleus changes in its staining reaction at this period, taking basic dyes more freely. Feulgen's method gives no chromatin reaction in this region however, only irregular threads and blebs of a deeper green are seen. The subsequent development shows that these threads gradually move from the periphery (Fig. 15) towards the interior (Fig. 16) until they have a ring-like appearance in section (Figs. 18, 19, 20), but in reality they completely enclose the karyosome or its remains.

The karyosome at the same time thins out. Fig. 16 shows openings at two places, probably points at which it ruptures, but possibly orifices through which the intrakaryosomic body passes out. At any rate, the alveolate outer rim disappears, and all that is left is the chromatinic mass imbedded in a little dense achromatinic substance with occasional remnants of the karyosome proper (Fig. 17). At about this period the parasite itself becomes free of its enveloping host cell, and the same alteration in staining reaction occurs as described in female organisms. This phenomenon is never seen until the disintegration of the karyosome is almost complete; but it may not happen even then, as very many "ring" forms are found still enclosed in host cells. The next step is the passage of the former central chromatin body towards this ring (Fig. 19), into which it appears to pass (Fig. 20); the component granules of this body fuse, if they have not already done so, to form a single irregular hollow spherule, or almost compact body. Haematoxylin staining of such stages as Fig. 20 gives the appearance of a dark ring of uniform structure; that is, this method does not reveal the presence of the larger chromatin body in the ring. I consider that one is always present, though veiled by the intense staining of the whole region. Meantime the chromatin granules, as shown by Feulgen's method, increase in number (Figs. 19, 20, 21). The last drawing (Fig. 21) is from a parasite preparing for the first nuclear division. The nucleus has passed to the surface and the chromatin granules and blebs have increased in number, though one much larger mauve structure is seen which is undoubtedly derived from the former intrakaryosomic body. In median section the ring-like form is still apparent, though not so definite, for processes are being given off, which are possibly the forerunners of the chromosomes, or the loops from which they are later constructed. The beginning of this process is shown in Fig. 21, while in Fig. 22 long coiled threads are seen, formed of densely stained achromatinic material containing numerous chromatin granules. This drawing is from a single section stained by Feulgen's method, and shows most of the nucleus; the rest of it, with about four irregular loops of chromatin, was in another slide stained with iron haematoxylin where the threads were stained homogeneously dark. There is some evidence that a longitudinal division was beginning.

The parasite drawn in Fig. 23 was stained with haematoxylin, and is of a rather later stage, probably the prophase of the first division. The threads are lightly stained and have an irregular outline but are not seen to contain darker

granules. Owing to their diffuse form they can hardly be called chromosomes, nor can the number be counted. The peculiar feature of this figure is the small but quite distinct karyosomic remnant. Such bodies were seen in a few other parasites. It might be suggested that these are really macrogametes or early sporogonic stages, but the general appearance, particularly of the chromatinic threads, contradicts this, and also the parasite figured here was fixed in September when, as mentioned above, no sporogonic stages were present. The karyosomic structure is probably derived from the protoplasmic mass in which the chromatin granules were previously embedded—sometimes quite a large body.

The nucleus of the male parasite now divides, and its further development will be described in Section (iv).

An examination of Foulon's Figs. 9–14, 16, 19 and 20, shows an almost complete reversal of the interpretation which I would put upon them; that is, what he calls development of the macrogamete I call development of the male previous to the first nuclear division, and *vice versa*. Now his whole argument depends on the fact that he finds no karyosome or remnant of it in the macrogamete (incidentally what he considered the macrogamete is really the zygotic spindle stage) or in other post-fertilisation stages; whereas I find that a karyosomic body is usually present in the zygotic spindle nucleus, and is invariably present and of quite large size in the macrogamete. Thus I consider that Foulon's Figs. 16, 19 and 20, show stages in the development of the female parasite and not the male as he says.

Nuclei, in which the karyosomes had disappeared and a hollow sphere of darkly stained material was present, "ring forms" (Foulon's Figs. 13 and 14 and my Figs. 18–20), are abundant, and I soon recognised them as being characteristic of sexual differentiation, but it was not till after the complete development of the macrogamete was elucidated that such stages were definitely established as belonging to the male cycle. Granted any one stage, the others follow from it (or precede it, as the case may be) in a perfectly clear and logical manner; indeed the steps are shown by both Foulon and myself in just the same order. Thus I consider that Foulon's Figs. 9–14 show stages in the male cycle and not in the female as he stated.

Foulon considered that in the development of the male (maturation of macrogamete in my reckoning) the large inner portion of the compound karyosome is extruded *en masse*. This I have not seen, and though the intrakaryosomic chromatin body and the achromatinic mass in which it is embedded are in some way ejected from the outer part, I do not think that such a large portion of the karyosome is extruded as is shown in Foulon's Fig. 17. The only comparable structures which I have seen appear to be artifacts produced by rupture of the karyosome in cutting rather thin sections (5 or 2·5 μ). There is no doubt that Feulgen's method of staining is of great help in elucidating the structure and development of the mature macrogamete, and of the male up to the time of the first nuclear division.

(iv) *Microgametogenesis*

When the nucleus of the male parasite has reached the periphery it proceeds to divide. Judging by the comparative rarity with which this and the three subsequent divisions are met with, they are passed through rapidly, with only a short resting period between them. No cyst wall is formed at this or a later period, though the pellicle may be somewhat thickened.

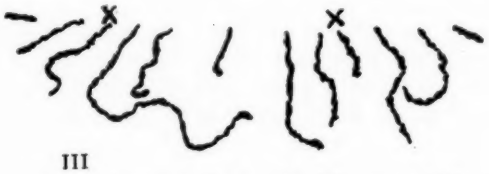
There is considerable variation in the appearance of the early division figures; as well as perfect bipolar divisions, structures are seen which have the appearance of multipolar and of amitotic divisions. These will be discussed in Section V, where it will be shown that such structures are possibly only phases in a modified form of ordinary bipolar mitosis. Fig. 24 shows this first division; chromosomes are present but have not stained intensely, and it was impossible to count the number. Though the figure suggests a multipolar division, it is more probably an anaphase, as will be explained below (p. 425).

An "amitotic" division is shown in the next figure (Fig. 25); a good many others, corresponding with this figure in lack of distinct chromosomes, were found. It is improbable that they are abnormalities, for they were obtained in renal organs containing excellently preserved material, many stages of which possessed perfect mitotic figures.

A "multipolar" division is drawn in Fig. 26 and is a reconstruction from two consecutive sections, each containing one complete anaphase figure. Altogether four such structures were present, so that eight nuclei would have been formed at the completion of this division. One anaphase group lay above and between, and another below and between the two depicted, but all were linked together at the centre. The chromosomes are stained densely and show a tendency to break into beaded structures. The surrounding nucleoplasm is very lightly stained and is clearly marked off from the cytoplasm. Both these features are characteristic of the nuclei of precytomere stages. Parasites containing eight nuclei are rare, as are also those showing the next division (fourth). No drawings are reproduced, as both dividing and resting nuclei of these stages are very similar to those shown in the next two figures (Figs. 27 and 28) of parasites undergoing or having undergone the fifth division. This is the last nuclear division before separation of the cytoplasm into cytomeres, and is often observed, while the subsequent condition in which approximately 32 resting nuclei are present is relatively abundant.

Some of the nuclei of a parasite during this fifth division are shown in Fig. 27 (all the other nuclei were in late anaphase). The chromosomes are very clear, and are characteristic of the precytomere condition (tendency to form beads). In many of the nuclei it was possible to count the chromosomes. Six are seen at either pole, but the difficulty of making a count is greatly increased because each chromosome is looped back at the pole, *i.e.* has a longer or shorter tag, so that it is **V**- or **J**-shaped. Hence to ascertain the number of chromosomes it is necessary to count all the free ends and divide the result by 2. In some

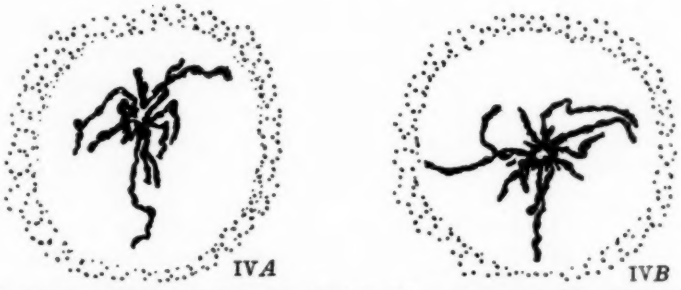
cases it was possible to make separate drawings, with the *camera lucida*, of each of the chromosomes at a single pole; but at best this is a difficult task, and does not give very trustworthy results. Whenever it is possible to count the chromosomes at all, however, 12 ends are present at each anaphase pole, and at least some of these ends are united in pairs at the polar extremity; hence it is reasonable to suppose that the other pieces are similarly linked, and that therefore 6 chromosomes are present. (Occasionally I have seen figures which appeared to have 7 chromosomes.)



III

Fig. III. September. Bouin, stained Giemsa. ×2000.

IV*A*^{1}



IV*A* IV*B*

Fig. IV. October. Sublimate acetic. Feulgen's stain. 7·5 μ. ×2000. *A* and *B*, polar view of two nuclei; *A*^{1}, individual chromosomes of *A*. Explanation in text.

The 12 isolated ends of the chromosomes of Fig. 27, *A* are shown at Fig. III. Those marked *X* were joined together, but the other connections could not be drawn with accuracy. Fig. IV, *A* and *B*, show two nuclei at the same stage, and the 6 chromosomes of *A* are drawn separately at Fig. IV, *A*^{1}.

The resting condition following this fifth division is shown in Fig. 28. The structure of the nuclei is very characteristic. Each is composed of a compact central mass of deeply stained chromatin, often in distinct beads, surrounded by a fairly large volume of nucleoplasm, which stains, if at all, only with non-basic dyes.

At an early date in these investigations many examples of this stage were noticed. Now the number of nuclei in these forms is approximately 32 (never more than 40), and there are also about 32 nuclei all told when the male has divided into uninucleate cytomeres; hence it was suspected that the former stage directly preceded the latter. The discovery of a periodic development confirmed this suggestion; for, as stated in Section III, at certain times of the year no stages of sporogony occurred, and it was at this very time that parasites with about 32 "compact" nuclei and also forms composed of uninucleate cytomeres were most abundant.

The division of the male into a number of cytomeres before the development of gametes is one of the bases for the classification of *Merocystis* in a separate genus of the family Aggregatidae. It is, however, found in *Myriospora trophoniae* (Lermantoff, 1913), and something similar was seen in *Caryotropha mesnili* (Siedlecki, 1907) which is a genus usually placed in a separate family (cf. also *Dorisiella scolelepidis*: Ray, 1930).

Foulon gives a figure (Fig. 30) showing separation into uninucleate cytomeres, but owing to scarcity of this stage he was not certain whether cleavage is always round a single nucleus. Dakin also portrays (Fig. 6) the formation of septa, but considers it a stage in sporogony. I have found 50 or more parasites in which cytomere formation is occurring, or has just taken place, and each cytomere has always 1 nucleus within it (30–40 such nuclei altogether). Fig. 30 is a surface view. Cytoplasmic division begins at the surface, and internally separation is at first incomplete. The nuclei in these cytomeres are always in a similar condition—*i.e.* the chromatin is in the form of lightly stained and irregular threads or blebs forming an indefinite network extending throughout the whole nucleus, which appears large, but is really about the maximum size of the nucleus of the precytomere phase. The vast difference in structure between nuclei before and after this cytomeric cleavage makes it perhaps difficult to believe they are consecutive stages, but not only have we the evidence given above, but also that derived from such forms as shown in Fig. 29. This must be regarded as an abnormality, but is nevertheless useful in showing that some parts are cut off into regions, each containing a single diffusely stained nucleus, while the rest of the cytoplasm is undivided, and contains a number of nuclei, either in mitosis or in the resting state, whose structure is typical of the precytomere phase, and is identical with that shown in Fig. 28. Occasionally stages are found in which the cytomeres contain a single dense nucleus (Fig. V, *F*), and also precytomeric forms with diffuse nuclei. These may be abnormalities, but more generally are transition forms. The rarity of transition phases suggests that this alteration in nuclear condition occurs rapidly.

Nuclear division takes place shortly after the formation of the cytomeres. It is usually an accurate mitosis, in which 6 chromosomes can be counted at each pole, as in Fig. V, *E*. Fig. V, *A* and *D*, show metaphases, and *B* and *C* early anaphases, characteristic of this species, and of a peculiar type, which

will be discussed below. Subsequent resting stages show exactly the same structure as those of the uninucleate cytomeres, *i.e.* "diffuse" nuclei. Later, when the nuclei become plentiful, they become progressively smaller and stain more densely, but they never have the "compact" precytomere form, except as abnormalities.

The later divisions seldom occur synchronously, as can be seen in Fig. 31; and though generally mitotic, with apparently 6 chromosomes in the ana- and

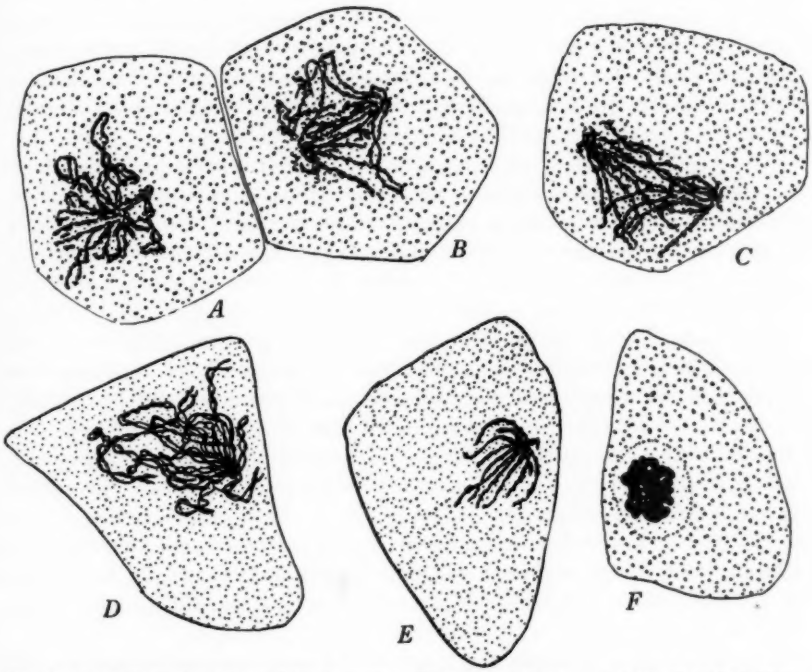


Fig. V. *A*, *B*, *C*, September. Sublimate acetic. Feulgen's stain. 5 μ. ×1500. *D* and *E*, May. Bouin, iron haematoxylin. 5 μ. ×1500. *F*, November. Sublimate acetic. Feulgen's stain. 7·5 μ. ×1500. Explanation in text.

telophasic poles, such forms as are seen in Fig. 32 are not uncommon. These appear to be amitotic, with the chromatin drawn out into thread-like structures having little resemblance to chromosomes.

A feature of interest is the persistence or disappearance of the nuclear membrane during division. Before cytomeric cleavage it is invariably present, but not afterwards; and though generally present in the first division, after the second it becomes increasingly rare.

Divisions continue until a very large number of nuclei are formed which are at first not confined to the surface, but by the end of the process are all so placed. Right up to the final division bipolar mitotic figures are seen, but the

chromosomes are so tightly packed together that it is hard to distinguish the number. It is, however, certainly more than 3, so there is no justification for supposing that a reducing division occurs at this time. After this final division (Fig. 33) the nuclei are all lying close to the periphery of the cytomeres, and are composed of a densely stained coarse irregular network. In section each nucleus has a rather square though ill-defined outline, very different from the earlier ones with their complicated mass of fine threads surrounded by the more or less spherical membrane. The last stage is the contraction of each nucleus, into a compact densely stained body, pear-shaped or oval: each of these is a microgamete. At the tip it appears to bear a small cap of achromatinic substance, which can sometimes be observed in haematoxylin or Giemsa preparations, but nearly always after Feulgen's method (as in Fig. 34, where the cap is greenish). Foulon does not show this cap.

No flagella were observed either in living material or in smear preparations. I do not think they are present, but in view of the difficulty found by other investigators in demonstrating these structures in other Coccidia it is possible I have overlooked them. It will be noticed that the microgametes depicted are rather unlike those of the majority of the Eimeriidea, in that they are much broader and shorter, and in fact resemble more those shown by Naville in *Klossia helicina*—one of the Adeleidea. Once the gametes have formed, the pellicle which has heretofore enclosed the cytomeres breaks down, and the gametes are set free from the residual protoplasmic mass and pass out into the cavities of the renal organ where isolated ones are frequently seen. In this position it is often difficult to distinguish them from the leucocyte-like cells which possess very dense nuclei and little cytoplasm, though this can be revealed by counter-staining.

As Fig. 33 shows, the development in some cytomeres may precede that of others in the same organism, so that cytomeres may sometimes be released in an immature condition. Usually this does not happen till the gametes are ripe.

A discussion of Foulon's interpretation of early microgametogenesis will be given at the end of the section on Sporogony. With his findings on the later stages after the cytomeres have been formed I am in agreement, except for such points as are mentioned.

(v) *Fertilisation and first division of zygote*

The actual penetration of the microgamete into the macrogamete I have never seen. This is unfortunate but not very surprising; for the process is probably very rapid and there is little hope of seeing the small non-flagellate gamete, composed almost entirely of chromatin, passing into the macrogamete, in which, to add to the difficulty, the chromatin network is lying at the surface. However, by comparison with similar stages in other Coccidia and gregarines, in which fertilisation has been seen, there is no doubt as to when it occurs (*i.e.* after the formation of what has been described above as

the macrogamete, and before the development of the typically spindle-shaped nucleus). The macrogamete—to recapitulate—is free in the cavity of the renal organ, and is surrounded by a firm but not thick pellicle, and has a somewhat elongated nucleus, one end of which comes very close to, or even touches, the surface of the parasite. The chromatin, which by this time has formed a densely stained, much coiled, and interlocked network, also abuts at this point. The nucleus contains a fairly large karyosomic body, devoid, however, of chromatin.

Once the microgamete has entered, a double-layered fertilisation membrane is laid down, which persists throughout the whole sporogonic cycle, and is the same structure as the cyst within which the spores are ultimately developed—no further thickening occurring. The membrane tends to separate from the parasite, and sometimes to be thrown into folds. This is more evident in later stages, but may occur quite early. The folding is probably an artifact, as living parasites never show it.

This membrane is seen in Fig. 35, which shows, I consider, a parasite soon after fertilisation. The chromatin nearest the surface is still in a condition similar to that of the macrogamete, *i.e.* so densely coiled that it appears granular, while towards the interior it has already formed the double thread, common in later stages. Fig. 36 shows the same coiled network close to what is, I think, the cone of reception (a structure only seen in a few other very similar stages), which consists of almost homogeneous cytoplasm showing faint striation. A number of long fine chromatin threads, enlarged here and there into blebs, originate near the central mass, and pass out from it to the nuclear membrane of the opposite pole. Unlike those of the prefertilisation cobweb stage, these threads are not usually attached throughout their length to the nuclear surface, but pass through the space to terminate there. No karyosome is present in this figure, but one is present in other parasites at this stage. The nucleus is not greatly elongated. Fig. 37 shows a parasite in which the "spindle nucleus" stage has been reached. Here a complicated intertwined mass is present near the periphery, and passing from it are some fine threads terminating as before on the nuclear membrane, but many appear to have cohered to form a thicker fibrous structure (the rupture in the centre is the line where the two obliquely cut sections meet). A large karyosome is present.

In Fig. 38, no cohesion of the threads has occurred. They are fine and have been drawn with the greatest possible accuracy, though they do not, as the drawing suggests, all lie in one plane. Some are attached to the nuclear membrane for their whole course, the majority only terminate there. Though this picture shows a more complicated condition than is usual, somewhat similar stages are not uncommon. The threads are very densely stained, the larger ones appearing multiple, as they often divide and then reunite with one another, or with other threads. They have a smooth outline and remarkably straight course. Sections cut across the nucleus of parasites, like those in Figs. 38 and 39, have a very characteristic structure. The chromatin threads show as clear round bodies, but on focusing can be seen to be parts of elongated fibres.

Sometimes they pass through the nuclear membrane into the cytoplasm at the pole away from the surface. The long axis of the nucleus passes along a radius of spherical parasites, and is at right angles to the long axis of elongated organisms (the latter is the more usual condition). Fig. 39 shows the chromatin in the form of a long double thread, probably due to cohesion of previously dissociated filaments (seen in Fig. 38). Though this interpretation seems most reasonable, it may be that the spindle nuclei pass into this condition directly after nuclear fusion, without an intermediate stage in which numerous fine threads are present. There is a danger in seeking to form a developmental series from such figures, when really there is no proof that they are not all different manifestations of the same condition. In any case a comparatively large number of spindle nuclei have the chromatin in the form of a few threads, very often two, which lie close together, and may join for a distance, but though more than two threads may be present, a complete single one has never been found.

All Coccidia whose life histories have been carefully investigated show such spindle nuclei following fertilisation. Foulon figured such a nucleus (Fig. 47) but considered it a prefertilisation stage chiefly because it is often found with the nuclear membrane not touching the surface of the parasite; but the spindle nucleus is not fully formed for some time after fertilisation and during this period it may retract somewhat from the membrane. Considering the detailed investigations made on many Coccidia, and the resemblance of *Merocystis* to other species, I have no hesitation in saying that fertilisation precedes the formation of the spindle nucleus, and this itself is a preliminary to the zygotic division.

The retraction of the chromatin to form the chromosomes of the zygotic division must be very rapid. The first stage I have seen is shown in Fig. 40; in this, finger-shaped processes of densely stained chromatin, probably chromosomes, are present. This is a reconstruction drawing, in which most of the chromatin appeared on one section, but a few portions were on the sections before and after, and it is therefore difficult to make an accurate count of the number of processes. There are however at least 10, and not more than 16 (therefore somewhere about the diploid number). It will be seen that the nuclear membrane persists, is somewhat elongated, and comes close to the surface. Zygotic division figures are rare, rarer indeed than any other stages. Fig. 41 is a peculiar one, but as it is one of two or three almost identical parasites, it is shown. The chromatin is in the form of short thick processes, possibly chromosomes, whose number it is impossible to count. The peculiarity of this, and others like it, lies in what seems to be an achromatic spindle—the only stage in which such a structure occurs—which is not found in all zygotic divisions, so may be an artifact. It is the only figure which even suggests a metaphase such as is found in the Metazoa, and many other Protozoa. Fig. 42 shows the separation of the paired chromosomes in the reduction division. No accurate count of the chromosomes can be made in this drawing, as it is re-

constructed, and probably some of the double structures should be connected, giving a lower count. There is nevertheless much indirect evidence that this is a meiotic phase. Obviously these doubled processes are formed either by lateral connection (partial or complete) of the chromosomes, or are due to longitudinal splitting of single pre-existing ones, in the former case part of a meiotic division, in the latter the metaphase of a mitotic one. Now the splitting of the metaphase chromatin loops during mitosis in *Merocystis* differs greatly from that seen in this figure (see below, p. 425). Briefly it occurs in long thin coiled threads, not at all like the short and plump structures seen in this figure. The chromosome number in both microgametogenesis and sporogony is 6, and no reduction division has been seen in the formation of the gametes, so the only stage where it is likely to occur is in the zygote; here the processes which lead up to division are very peculiar (particularly the spindle nucleus) and clearly distinguishable from all other nuclear divisions. There is every probability that the same type of chromosome cycle is found in all Coccidia and gregarines, and in *Merocystis* there is nothing to suggest that this is not true; on the contrary there is definite indication that reduction takes place in the zygote. I hope to obtain more material with such stages during the coming year.

It is worth noting that the coiled bodies seen in this Fig. 42 resemble the pictures of zygotic reducing divisions shown by Naville in numerous forms (*Aggregata*, 1925, *Klossia helicina*, 1927*b*, and *Urospora lagidis*, 1927*a*: see his review, 1931). Such paired and coiled chromosomes he compares with those of the zygotaene stage in Metazoa. Neither Dobell nor Bělař depict such coiled chromosomes in *Aggregata*, although the whole process of reduction, including stages in which the diploid chromosome number could be counted, was fully described by Dobell (1925).

(vi) *Sporogony*

As a result of the zygotic division two daughter nuclei, such as seen in Fig. 43, are formed. Not many (about six) of these stages were observed, but they were all very similar. The two nuclei are placed on the same side of the parasite, and are very lightly stained, the chromatin being scattered throughout the entire nucleus in the form of fine rods and granules which stain mauve with the nucleal reagent. These nuclei resemble those of resting stages in early sporogony, and differ greatly from those of the precytomere gametogenesis—being more like those of post-cytomere stages. They were not seen in any material fixed in September, when early gametogenic phases were abundant, and parasites containing them are always surrounded by a thick wall, so there is no doubt that they belong to the sporogonic cycle.

The second division of the sporont is shown in Fig. 44 and the isolated chromosomes of the poles marked A^1, A^2, B^1 are drawn in Fig. VI. The chromosomes are exceptionally darkly stained and firmly outlined; and at A^1 particularly, it was comparatively easy to see the 6 separate looped chromosomes.

Foulon said that the sporogonial divisions are of a less perfect type than those of the male cycle, but this I do not find true. In both it is possible to count 6 chromosomes, but this can be done more readily in the divisions of the sporont than in microgametogenesis, where the tendency of the chromosomes to form beads may confuse the appearance. The nuclear membrane, as shown in Fig. 44, persists; within, the nucleoplasm is finely granular. There is some indication that the chromosomes of one pole correspond in size and form with those of the other, and there is certainly a very long chromosome at either end.

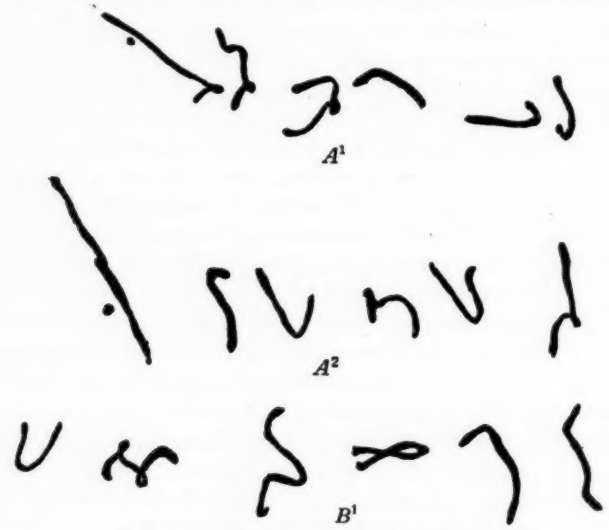


Fig. VI. ×1250. Explanation in text.

Fig. 45 shows a very complicated form of "multipolar" mitosis. The entire nuclear contents of the organism are portrayed, but owing to the complexity the only part that is accurately drawn is that marked *A*. Altogether four complete anaphase figures are present, but all are linked together. A parasite whose nucleus appears to be undergoing a very irregular division is seen in the next figure (Fig. 46). This and the previous division will be discussed in Section V.

Karyosomic bodies are occasionally found in nuclei of these stages. Figs. 47, *A*, *B*, *C*, are drawn from the same individual, in which two other anaphase mitoses, like *C*, were also present. Six chromosomes could be counted with certainty at the anaphase *C*, but in the multipolar division *A* there are more than 6 threads terminating at each pole; in fact the whole structure, though not so complicated, resembles Fig. 45, and both will be referred to below. These multipolar divisions show some similarity to Figs. 25 and 26 in Foulon's paper, though he regarded them as belonging to the microgametogenic cycle. His chief reason for this opinion was the presence of karyosomic remnants, but,

as I have already shown, it is in the sporogonic cycle that these bodies are most likely to be present. I therefore consider that Foulon's Figs. 25 and 26 belong to the sporogonic cycle. As to his Figs. 50 and 51, which he regards as the first sporogonial divisions, I think they belong to the male cycle, because of the rather compact chromatin figures with irregular beaded threads (chromosomes?).

Nuclear divisions continue rapidly, but they cease to be synchronous, as is shown in Fig. 48, *A*, *B*, *C*, where 3 out of about 20 nuclei are drawn. The usual chromosome number is found in the anaphase *C*. The chromosomes here are characteristic of this and other sporogonial divisions, and have slightly irregular but continuous outlines, never beaded like the precytomere chromosomes. Fig. 48, *A* shows what I think must be regarded as an anaphase in which precocious splitting of the chromosomes preparatory to the next division has taken place (as will be explained below). The resting nucleus (Fig. 48, *B*) is noticeably different from those of microgametogenesis with a corresponding number of nuclei, the chromatin being in the form of small rods and granules disseminated throughout the entire nucleus, with no central aggregation. The nuclei on a surface slice of a parasite containing in all about 50 to 60 is shown in Fig. 49. One of these (*A*) is drawn enlarged in Fig. VII, where 6 looped chromosomes can be distinguished. *B* and *C* represent early anaphases.

Fig. VII. ×2000. Explanation in text.

The next figure (Fig. 50) shows a very characteristic appearance of the nuclei in a sporont containing over 60. It will be noticed that many have a bridge-like shape suggesting an amitotic division. Like other "amitotic" forms there is probably another explanation which will be mentioned below. Side by side with these forms are telophases and anaphases of the usual type. The similarity of these "bridge-like" nuclei to those shown by Foulon (Fig. 28) is obvious, and leads me to believe that his drawing is from a parasite of the sporogonial cycle and not a stage of microgametogenesis as stated. In any case, judging by the proximity of the nuclei in his drawing, there would have been many more than 30–40, so that it could not belong to the male cycle, as cytomeric cleavage would have taken place. Foulon's Figs. 52 and 53, supposed to depict stages of the sporont, show nuclei each with a central dense and beaded chromatinic mass, surrounded by much achromatinic substance. They are identical with my Fig. 28, and I regard them as stages in microgametogenesis just before cytomeric segmentation. In fact Foulon has exchanged early sporogony with early microgametogony. I would possibly have

given the same interpretation, had it not been that the periodicity of development rules it out as being quite incompatible with observations made particularly on material fixed in autumn.

Prophase (*A*), metaphase (*B*), and early anaphase (*C*), from a parasite with many nuclei are shown in Fig. VIII, but will be discussed in the next section. As the nuclei become increasingly numerous, they also become smaller, more compact and densely stained, until they appear as in Fig. 51. This is a surface view, and, as before, all the nuclei lie near the periphery. Owing to the small size of the nuclei, the chromosome number is hard to ascertain, but it is probably 6, and certainly not 3; this is true for all these stages and presumably also for the final one. In the parasite shown, dumbbell-shaped (and one tripolar) "amitotic" structures are present as well as mitotic figures.

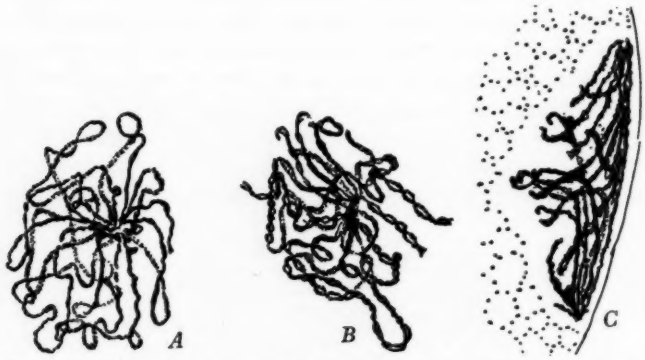


Fig. VIII. January. Sublimate acetic, Feulgen's stain. 7·5 μ. ×2666. Explanation in text.

After the final division the nuclei contain very dense chromatin networks, and are subspherical or even cubical, as they are very closely pressed together. Fig. 52 does not fully indicate how numerous they are at this time. There is now a sudden change in shape (Fig. 53), the nuclei becoming pear-shaped, or triangular when seen in section, with the apex orientated almost invariably towards the cyst wall. The nuclei have a more definite outline than in the previous stage, and this probably accounts for the fact that Dakin considered them as the male gametes. Such stages are abundant at certain times of year. Eventually there is a rapid folding of the surface at certain parts (Fig. 54), until finally the nuclei lie scattered rather irregularly throughout the protoplasm, though some remain at the surface. Sporoblastic cleavage is seen beginning in Fig. 55, and complete in Fig. 56. The nuclei retain their conical form during this time, and each lies at one pole of the sporoblast, the pointed end even projecting from the surface.

It is peculiar that Foulon gives no figures showing these conical nuclei, either on the surface or during their inward passage. He considered that they moved inwards while they were of the shape shown in my Fig. 52. He gives

one figure depicting this, but remarks on its rarity. (I do not find stages showing the folding of the surface very rare.) The further development of the sporoblasts to give two sporozoites is accurately described by Foulon, but there are a few points to add to his account.

The sporoblast, when first formed, has a single nucleus with a structure essentially similar to that of the conical nuclei, *i.e.* chromatin in a dense coarse network with a spongy appearance, which is retained during sporozoite formation, though it is not so apparent during division.

The protoplasm contains a peculiar dense protoplasmic body (Fig. IX, *A*) figured also by Foulon. When this structure makes its appearance is uncertain. Certainly it is not present in all sporoblastic stages, as none of the sporoblasts in Fig. 56 contained such a body. It seems to be derived from the cytoplasm and is well stained with haematoxylin, but with Feulgen's method it is stained more deeply than the rest of the cytoplasm by the counter-stain. The outline of this body is fairly definite, and its substance appears to be homogeneous, though haematoxylin staining sometimes shows darker granules (Fig. IX, *B*)

Fig. IX. *A–E*. ×2000. 10 μ. *A*, May. Bouin, haematoxylin. *B* and *C*, January. Sublimate acetic (*B*, stained haematoxylin; *C*, Feulgen's method). *D* and *E*, May. Bouin, haematoxylin. Explanation in text.

such as Foulon found. Presumably it divides later into two; for in the uninucleate sporoblasts generally only one is found, and in the binucleate forms two, but judging by the number of times when this condition is reversed, it may become doubled before, during, or after the nuclear division (see Fig. IX, *A–D*). There is no evidence that this body is derived from any of the usual cytoplasmic inclusions (mitochondria or Golgi bodies). The sporoblasts are at first only partly separated from one another, but in Fig. 56 are seen to be surrounded by a firm pellicle, which remains as the sporocyst but never thickens.

The one nuclear division which occurs is mitotic, and not amitotic as Foulon suggests. Short thick processes, probably the chromosomes of anaphase and telophase, are seen in Fig. IX, *C* and *D*. Fig. IX, *C* certainly seems to show chromosomes, and possibly 6, though this is not certain. The nuclei which result often remain connected for some time by a bridge of chromatin, which may give the appearance of an amitotic dumbbell. The two nuclei which are reconstructed have the same structure as those at the uninucleate stage. They then elongate and the cytoplasmic bodies also lengthen, and each becomes attached to one end of a sporozoite nucleus as a "tail" (Fig. IX, *E*).

Careful focusing nearly always shows such sausage-shaped bodies, and it is only when the sporozoites are cut transversely that the protoplasmic bodies appear spherical (Fig. X, *F*). It is probable that these cytoplasmic "tail-pieces" persist and are a fundamental part of the mature sporozoite. In many cases (Fig. X, *H* and *I*) no such parts could be seen, but this is probably an abnormality, due to degeneration or bad fixation. A residuum of very lightly stained protoplasm containing some of the usual refringent granules (present in all except the youngest parasites, but not described in this paper) is left at the centre between the sporozoites. The region between this and the spore membrane appears to be empty.

The fertilisation membrane is fairly easily ruptured by this time, and sometimes ripe spores (containing two sporozoites) are found free. Very rarely they have been found embedded between host cells (Fig. X, *I*), but isolated sporo-

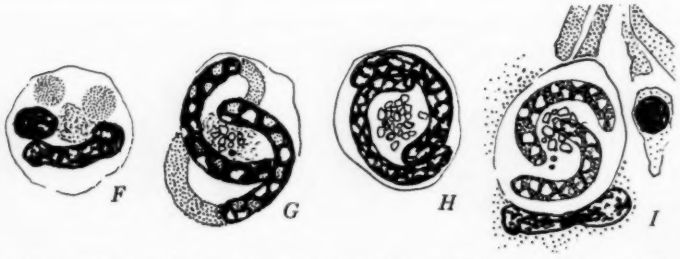


Fig. X. ×2000; cut 10 μ. Explanation in text. May. *F* and *G*, Bouin, haematoxylin. *H* and *I*, Bouin, Giemsa.

zoites have never been found. It is probable that this figure shows a spore in an abnormal condition and position. There is no evidence that sporozoites ever develop further in the kidney, and indeed such spores are probably degenerating (notice the pale coloration of the sporozoites).

As previously mentioned, in material fixed in June 1933, many degenerating cysts and isolated spores were seen. If I am correct in considering these bodies as derived from *Merocystis*, and their size and shape certainly suggest it, then many of the cysts are being attacked by, or are filled with, phagocytes, while the isolated spores are filled with material probably derived from the break-down of the sporozoites. The entire contents usually give the chromatin reaction with Feulgen's stain.

Abnormalities in the number of sporozoites found in spores are not very rare, 3 and 4 being most usual. I have never seen a spore containing only 1 sporozoite, which Dakin thought was the normal number.

V. Nuclear divisions in *Merocystis*

In both microgametogenesis and sporogony anaphase and telophase figures are abundant, and it is obvious that mitosis is the normal method of division. The form of mitosis is similar in both these cycles but differs from that usually

met with in most Protozoa and Metazoa. It falls into the category described by Bělař as "paramitosis" (1926*b*). I would like however to state that I had determined the main outlines of the process of division before I had seen Bělař's monograph, so that my findings were not originally influenced by this important work.

No centrosomes or centrioles with attendant asters have been seen, but I do not think they have been overlooked through over-differentiation in staining. Moreover, nothing resembling an achromatic spindle has been observed (with the possible exception of the first zygotic division), and nothing resembling the metaphase of an ordinary equatorial plate (again with the possible exception of the zygotic division).

In many nuclei, closely entangled double chromatin threads are present, with the two threads of each pair twisted round one another, or with the two strands running parallel for some distance and then twisting or separating. One such nucleus has been drawn with great care in Fig. VIII, *B*, p. 422, but somewhat similar ones can be seen in other drawings (Fig. V, *A* and *D*, p. 415). I regard these as showing the longitudinal splitting of the chromatin, which seems to occur while it is still in the form of a continuous thread, only later dividing into a number of pairs of isolated threads—the chromosomes (Fig. V, *D*, p. 415). Owing to complications and twists of the threads it is impossible to count the number with any certainty. There is no contraction and concomitant thickening of the chromosomes in the metaphases, like that found in most forms. In the early anaphase (Fig. V, *B* and *C*, p. 415 and Fig. VIII, *C*, p. 422) one end of each of the very long chromosomes of a pair passes to either pole, but for a considerable time the greater length of the chromosome remains coiled round its fellow, and as the attachment of the chromosomes is not really terminal (each is looped back in a **V** or **J** shape), the appearance of the threads at this time is very complicated. Another point to be mentioned is that the "metaphase" does not necessarily lie in the equatorial region of the division figure. The chromosomes pass out at an angle from it towards either pole, hence the production of figures which may suggest a multipolar division (Fig. V, *C*, p. 415). Foulon made no mention of prophases or metaphases though he figured a few anaphases with definite chromosomes. He considered that there were probably 6 chromosomes, but the figures in which he depicted them are all from stages of microgametogenesis after cytomeric division (his Figs. 32–39) and zygotic division (Fig. 49) and in only two of these (Figs. 32 and 36) is it possible to count the number (in Fig. 32 there seem to be only 4 looped chromosomes at each pole). No definite chromosomes are shown in divisions of precytomere stages or of the sporont (his figures should however be reversed), but he considered these to be of a less perfect type.

The next questions to be considered are whether the multipolar figures seen really represent multiple divisions, and whether amitosis occurs.

Multipolar divisions are by no means uncommon, and Figs. 26, 45, 47, *A* show some. Fig. 26 is one in which the haploid chromosome number appears

to be present at each anaphase pole, though there is a common chromosomal connection between the four complete figures (only two show). More than the haploid number, however, is certainly present at each pole of the multipolar forms in Figs. 45 and 47, *A*. The thread-like chromosomes emerging from the polar ends are mostly in pairs, the partners of a pair being often entwined in a manner resembling the above-mentioned metaphase. I regard them as being essentially similar to the latter; since they show the splitting of the chromosomes preparatory to the next nuclear division, so that if the organism had completed its division, 8, not 4, nuclei would have been reconstructed in Fig. 47, *A*; while in Fig. 45, it would have been 16, and not the 8 which are already indicated by the polar grouping of the chromosomes. If this interpretation is correct then such a bipolar form as is seen in Fig. 48, *A* is really preparing for the next division, as is indicated by the pairs of twisted chromosomes. (All the threads are not drawn owing to the confusion it would have caused.) Before discussing the multipolar forms further, it is necessary to mention the apparently amitotic nuclei (Figs. 25, 32, 46, 47, *B*, 50, etc.). Bělař has stated that nuclei with bridge-like connections—seen in *Aggregata* and other forms with the "paramitotic" type of division—are really due to the fact that owing to the great length of some of the chromosomes, reconstitution of the daughter-nuclei into the resting condition occurs before severance of the longer chromosomes is complete, hence the interphasic nuclei remain connected and give the appearance of amitosis. This seems very plausible, and is probably the explanation of such forms as are seen in *Merocystis*. There is no very noticeable size-differentiation in the chromosomes of *Merocystis*, so it is quite possible that all might remain connected, or only slightly separated, and pass into the interkinetic phase in that position. Thus Figs. 32, 47, *B*, and 50, etc., would not be pictures of amitosis, but resting stages of bipolar divisions, and Fig. 48, *A* would be a subsequent metaphase.

The process might be carried further and would account for the multipolar figures. In that case they would not be multiple divisions at all, but only the end-result of a number of bipolar mitoses in which some or all of the pairs of chromosomes had failed to separate at the telophase. The process does not necessarily involve any suppression of the resting stage, which is really seen in the "amitotic" nuclei. I cannot say if this be the correct interpretation; it seems probable, and its convenience is obvious. It avoids the necessity of supposing that amitosis occurs, for it is difficult to conceive that amitosis and a perfect mitosis can both occur in the same individual.

VI. Summary

1. The life history of *Merocystis kathae* shows that development follows a seasonal course. The youngest stages may appear in the kidney any time between March and June. Growth proceeds and sexual differentiation takes place but mature gametes are not found until November, when the first stages

of sporogony also make their appearance. Mature spores containing sporozoites are formed in January and become increasingly common up to May. Degenerating stages of spores are common in summer.

2. The smallest parasites within the cells of the kidney are about 10μ. Growth occurs in the intracellular condition. The karyosome is complex, and contains a chromatinic body; in older parasites no chromatin reaction is given by the nucleus outside the karyosome.

3. Sexual differentiation is not perceptible until growth is almost complete.

4. In the female the intrakaryosomic body emerges, though a large karyosomic remnant remains until after fertilisation. Very fine granules of chromatin reappear in the nucleus, and form a fine network, which incorporates the intrakaryosomic body, and later forms the characteristic prefertilisation "cobweb" nucleus. This network contracts in the mature macrogamete. Meantime the parasite has become free of its host cell, and the cytoplasm stains more intensely.

5. In the male, the elongated karyosome breaks down, generally completely, leaving the centrally placed chromatin body surrounded by a ring (in sectional view), of deeply stained material containing granules of chromatin, into which the central body passes as in the female. The male is released from the host cell, and, as in the female an alteration of the cytoplasmic staining occurs.

6. The nucleus, in the male parasite, moves to the surface, and undergoes repeated divisions until approximately 32 nuclei are formed. Cleavage of the cytoplasm brings about the formation of uninucleate cytomeres; within each of these are produced many nuclei, which lie near the surface, and finally become the microgametes.

7. After the macrogamete is fertilised, a thick membrane is formed outside the zygote. The nucleus assumes the characteristic spindle shape and subsequently divides.

8. Nuclear divisions proceed, until finally a great number of cone-shaped nuclei are found on the surface. This becomes folded and the nuclei sink in, and each is surrounded by a portion of cytoplasm to form a sporoblast. A single nuclear division then occurs, and the two nuclei elongate to form sporozoites each with a small cytoplasmic body.

9. Nuclear divisions are mitotic, but the longitudinal splitting of the chromosomes occurs without contraction. It is probable that "amitotic" appearances are due to the incomplete incorporation of these long chromosomes at the telophase poles, and the multipolar divisions to the subsequent division of such "amitotic" resting nuclei. Six chromosomes can be seen at the anaphase poles, but each is looped back so that it is V or J shaped. This chromosome number is characteristic of the divisions of both microgametogenesis and sporogony.

10. There is good evidence that, as in other Coccidia, reduction division occurs in the zygote, and that this is therefore the only diploid phase.

I would like, in conclusion, to express my indebtedness to Prof. J. Brontë Gatenby for his unfailing interest and advice, and also to Mr Clifford Dobell, F.R.S., for his great kindness in reading my manuscript and making many useful suggestions.

REFERENCES

Bělař, K. (1926*a*). Zur Zytologie von *Aggregata eberthi*. *Arch. Protistenk.* **53**, 312.

—— (1926*b*). Der Formwechsel der Protistenkerne. *Ergbn. Zool.* **6**, 235.

Brasil, L. (1904). Sur une coccidie nouvelle parasite d'un cirratulium. *C.R. Acad. Sci.*, Paris, **139**, 645.

Dakin, W. J. (1911). Notes on a new coccidian (*Merocystis kathae* n.gen. n.sp.). *Arch. Protistenk.* **23**, 145.

Debaisieux, P. (1922). Note sur deux coccidies des mollusques: *Pseudoklossia* (?) *patellae* et *chitonis*. *Cellule*, **32**, 231.

Dehorne, A. (1930*a*). Sur l'*Aggregata* de *Nereis diversicolor*. *C.R. Soc. Biol.*, Paris, **103**, 665.

—— (1930*b*). Présence d'éléments du type sporozoite d'*Aggregata* dans les divers tissues des Polychètes. *Ibid.* p. 959.

Dobell, C. (1914). Le cycle évolutif de l'*Aggregata*. *Bull. Inst. océanogr. Monaco*, No. 283.

—— (1925). The life history and chromosome cycle of *Aggregata eberthi*. *Parasitology*, **17**, 1.

Dobell, C. and Jameson, A. P. (1915). The chromosome cycle in Coccidia and Gregarines. *Proc. roy. Soc.* B, **89**, 83.

Duboscq, O. (1918). *Selysina perforans* (Dub.). *Arch. Zool. exp. gén.* **58**, 1.

Foulon, C. (1919). *Merocystis kathae* Dakin une Aggrégate de *Buccinum undatum*. *Cellule*, **30**, 123.

Goodrich, H. Pixell (1914). The sporogony and systematic position of the Aggregatidae. *Quart. J. micr. Sci.* **60**, 159.

Hentschel, C. C. (1926). On the correlation of the life history of the Acephaline Gregarine *Gonospora*, with the sexual cycle of its host. *Parasitology*, **18**, 137.

—— (1930). On the correlation of the life history of the Acephaline Gregarine *Gonospora*, with the sexual cycle of its host. *Ibid.* **22**, 505.

Hoare, C. A. (1933). Studies on some new ophidian and avian Coccidia, from Uganda, with a revision of the classification of the Eimeriidea. *Ibid.* **25**, 359.

Jameson, A. P. (1920). The chromosome cycle of gregarines, with special reference to *Diplocystis schneideri*. *Quart. J. micr. Sci.* **64**, 207.

Kunze, W. (1907). Über *Orcheobius herpobdellae* Schuberg et Kunze, ein Coccidium aus *Herpobdella atomaria*, *Arch. Protistenk.* **9**, 381.

Lermantoff, E. (1913). Über *Myriospora trophoniae*, n.gen. n.sp. *Ibid.* **32**, 205.

Moroff, T. (1908). Die bei den Cephalopoden vorkommenden Aggregataarten (etc.). *Ibid.* **11**, 1.

Naville, A. (1925). Recherches sur le cycle sporogonique des *Aggregata*. *Rev. suisse Zool.* **32**, 125.

—— (1927*a*). Le cycle chromosomique d'*Urospora lagidis*. *Parasitology*, **19**, 100.

—— (1927*b*). Recherches sur le cycle évolutif et chromosomique de *Klossia helicina*. *Arch. Protistenk.* **57**, 427.

—— (1931). Les Sporozoaires (cycles chromosomiques et sexualité). *Mém. Soc. Phys. Genève*, **41**, fasc. i, p. 1.

Ray, H. N. (1930). *Dorisiella scolelepidis* n.gen., n.sp. *Parasitology*, **22**, 471.

Robertson, M. (1927). Notes on certain points in the cytology of *Trypanosoma raiae* and *Bodo caudatus*. *Ibid.* **19**, 375.

Setna, S. B. and Bhatia, B. L. (1934). On some gregarines from the prawn, *Parapeneopsis sculptilis*. *Ibid.* **26**, 34.

SIEDLECKI, M. (1898). Étude cytologique et cycle évolutif de la coccidie de la seiche. *Ann. Inst. Pasteur*, **12**, 799.

—— (1907). *Caryotropha mesnilii*. *Bull. Acad. Sci. Cracovie*, p. 453.

THOMAS, J. A. (1930). Sur le sporozoaire (coccidie) parasite de *Nereis diversicolor*. *C.R. Soc. Biol.*, Paris, **104**, 138.

VINCENT, M. (1927). On *Légerella hydropori*, a coccidial parasite of the malpighian tubules of *Hydroporus palustris*. *Parasitology*, **19**, 394.

WENYON, C. M. (1926). *Protozoology*. 8°. 2 vols. London: Baillière, Tindall and Cox.

WEDEKIND, G. (1927). Zytologische Untersuchungen an *Barrouxia schneideri*. *Z. Zellforsch.* **5**, 505.

DESCRIPTION OF PLATES

All drawings were made with the aid of a camera lucida. A Bausch and Lomb microscope with Zeiss apochromatic $\frac{1}{12}$" oil-immersion lens, and two different oculars were used. The date at which fixation of the material took place is stated in each case.

Abbreviations: B, Bouin's fixative; C, Sublimate acetic; H, iron haematoxylin; F, Feulgen's method; G, Giemsa's stain. R means reconstruction of drawing from more than one section and implies that the entire chromatin contents of the nucleus are drawn.

PLATE XI

Figs. 1–7. Young undifferentiated parasites, showing penetration of intrakaryosomic body—chromatin drawn as black granules or threads, non-chromatinic portions stippled. Hypertrophied host-nucleus seen in Figs. 1–4 and 7. All cut 5 μ. ×1700.

Fig. 1. May. C, H.

Figs. 2, 3, 4. May. C, F.

Figs. 5, 6. July. C, F. Nuclei only.

Fig. 7. July. C, F. Nucleus and part of parasite.

Figs. 8–14. Female parasites—nucleus and part of cytoplasm only. ×830. Figs. 9–14, parasites free of host-cell.

Fig. 8. December. C, F, 10 μ (greatest diameter of this organism 165 μ).

Fig. 9. December. C, F, 10 μ. *A*, achromatinic spherule; *B*, chromatin blebs. (*A* and *B* represent intrakaryosomic body.)

Fig. 10. January. C, F, 7·5 μ. Lettering as Fig. 9.

Figs. 11 and 12. November. C, F, 10 μ, R. Lettering as Fig. 9.

Fig. 13. November. C, F, 10 μ, R. "Cobweb" stage.

Fig. 14. January. C, F, 7·5 μ, R. Macrogamete. (Section containing karyosome stained haematoxylin.)

Figs. 15–23. Male parasites. Nuclei and part of cytoplasm only. Figs. 19–23 parasites free of host cells. ×830.

Fig. 15. September. B, H, 5 μ. Large oval karyosome, containing intrakaryosomic body.

Fig. 16. December. C, H, 10 μ. Network of achromatinic threads moving in to form "ring" round degenerating karyosome.

Fig. 17. September. B, G, 5 μ. Disappearance of karyosome.

Fig. 18. September. B, H, 5 μ. "Ring" form.

Fig. 19. December. C, F, 10 μ. A few chromatin granules appear in ring.

Fig. 20. December. C, F, 10 μ. "Intrakaryosomic" body passing into ring.

Fig. 21. September. C, F, 5 μ. "Ring" disappears, chromatin granules more abundant.

Fig. 22. January. C, F, 7·5 μ. Processes, which may be chromosomes, appear.

Fig. 23. September. B, H, 5 μ. Nucleus preparatory to first division.

PLATE XII

Figs. 24–34. Microgametogenesis. ×625, unless otherwise stated.

Fig. 24. September. B, H, 5 μ. Anaphase of 1st division.
Fig. 25. September. B, H, 5 μ. "Amitotic" nucleus.
Fig. 26. September. B, G, 5 μ. Two pairs of anaphase figures, all linked together in central region. ×1000.
Fig. 27. September. B, G, 5 μ. Nuclei of a parasite undergoing fifth nuclear division. ×1000.
Fig. 28. May. C, F, 7·5 μ. Nuclei in resting condition after fifth division.
Fig. 29. September. B, H, 5 μ. Abnormal parasite, some parts divided into cytomeres.
Fig. 30. January. B, H, 10 μ. Surface view of uninucleate cytomere stage.
Fig. 31. May. C, H, 5 μ. Section across organism after cytomeric division.
Fig. 32. May. B, H, 7·5 μ. A few cytomeres showing "amitotic" divisions.
Fig. 33. May. B, H, 5 μ. Last nuclear division completed.
Fig. 34. January. C, F, 7·5 μ. Achromatinic cap seen at tip of each microgamete. ×1000.

PLATE XIII

All figures ×625, unless otherwise stated.

Figs. 35–42. Fertilisation and first zygotic division.

Fig. 35. January. C, 7·5 μ. Reconstructed from two slides, one containing karyosome and some of chromatin thread stained H, other slide F.
Fig. 36. January. C, F, 7·5 μ, R. Cone of penetration.
Fig. 37. November. C, 10 μ, R. Outer part of nucleus stained F, inner part with karyosome H.
Fig. 38. November. C, F, 10 μ, R. Small karyosome present.
Fig. 39. May. C, H, 5 μ, R. Double chromatin thread.
Fig. 40. May. C, F, 5 μ, R. Chromosomes(?) of zygotic division.
Fig. 41. January. C, H, 7·5 μ. Metaphase of zygotic division.
Fig. 42. January. C, F, 7·5 μ, R. Pairing of homologous chromosomes in reduction division(?).

Figs. 43–45. Sporogony.

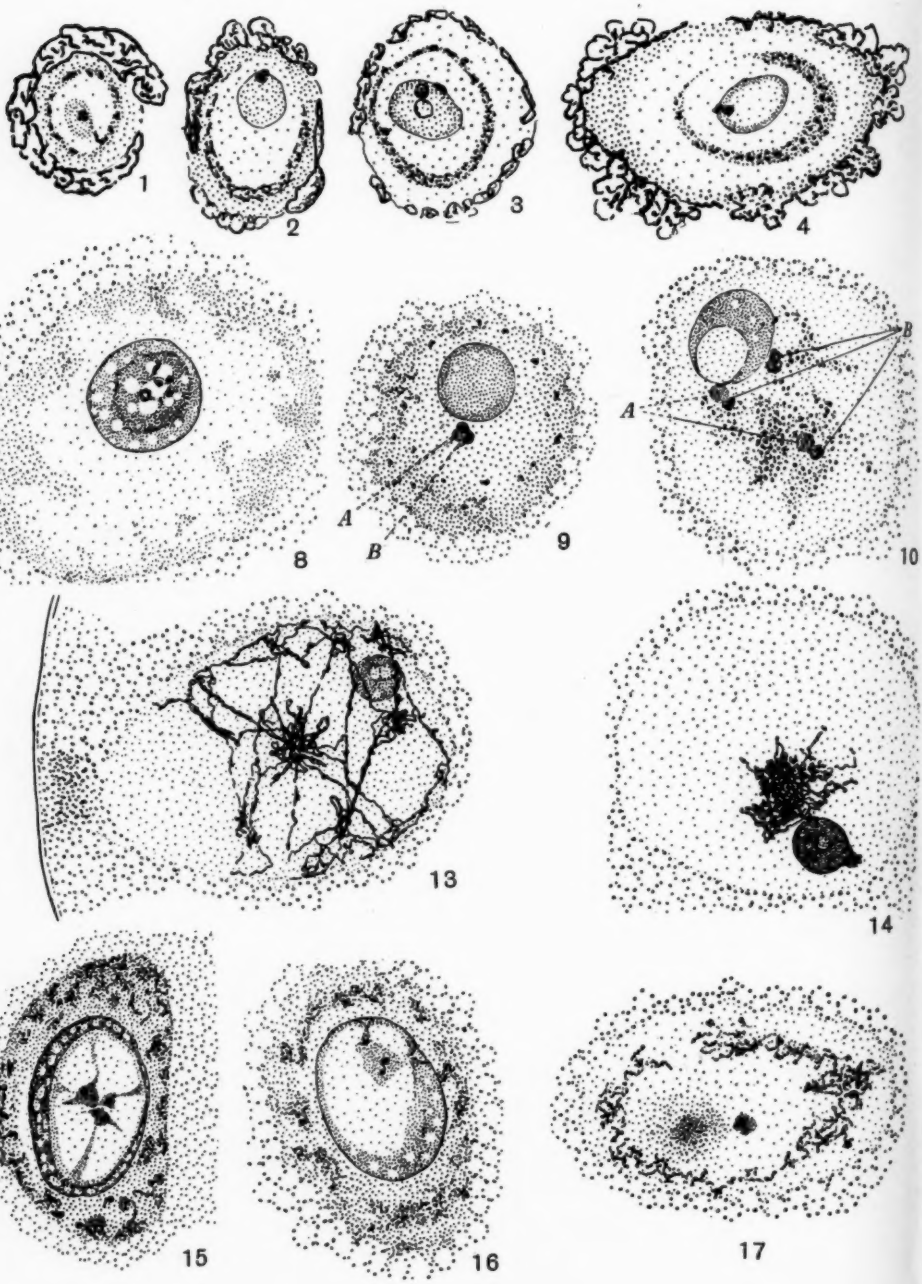
Fig. 43. January. C, F, 7·5 μ. Nuclei in resting condition after zygotic division.
Fig. 44. May. B, 10 μ, R. Second division. Anaphases marked *A* stained Giemsa; those marked *B* with haematoxylin.
Fig. 45. December. C, 10 μ, R. Portion near periphery stained F, internal portions H. Multipolar division: pole *A* drawn accurately. ×1000.

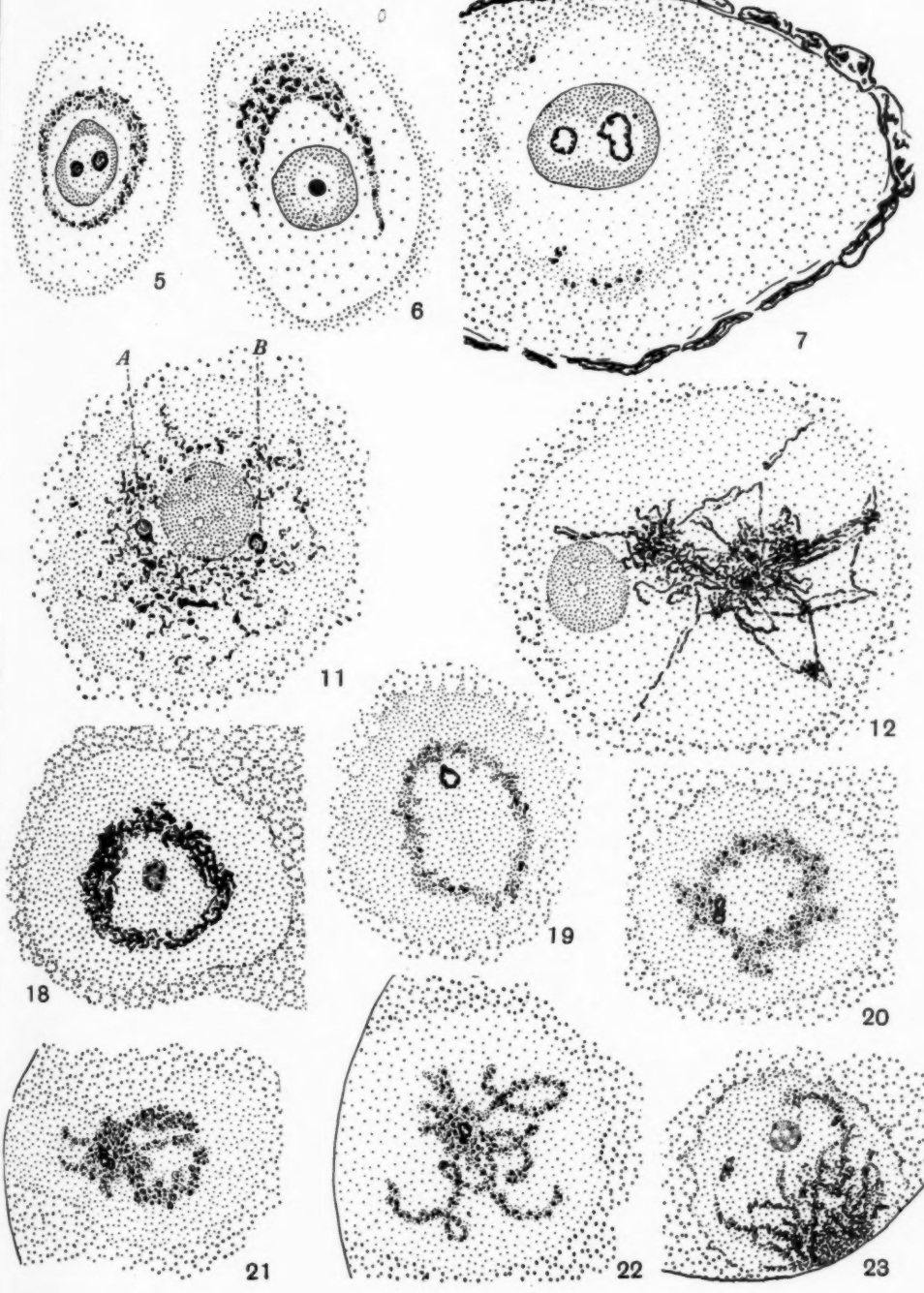
PLATE XIV

All figures ×625, unless otherwise stated.

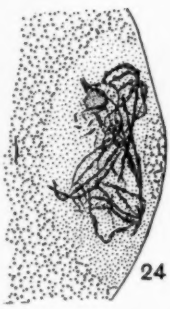
Fig. 46. December. C, F, 10 μ. "Amitotic" division.
Fig. 47. May. B, H, 10 μ. *A*, multipolar; *B*, "amitotic" division; *C*, anaphase pole with 6 chromosomes. ×1000.
Fig. 48. May. B, H, 10 μ. *A*, metaphase; *B*, resting nucleus; *C*, anaphase. About 20 nuclei, in all, in whole parasite. ×1000.
Fig. 49. May. B, H, 10 μ. Shows all nuclei seen in surface slice of parasite containing about 40 nuclei. *A*, late anaphase; *B* and *C*, early anaphases.
Fig. 50. May. B, H, 10 μ. "Bridge" nuclei.
Fig. 51. May. B, H, 10 μ. Surface view of parasite undergoing last nuclear division.
Fig. 52. May. B, G, 10 μ. Sectional view after last nuclear division.
Fig. 53. May. B, H, 10 μ. Nuclei have become conical.
Fig. 54. January. Sublimate formol, G, 7·5 μ. Folding of surface; sporoblastic cleavage.
Fig. 55. June. C, H, 7·5 μ. Segmentation into sporoblasts beginning.
Fig. 56. May. B, H, 10 μ. Segmentation complete.

(*MS. received for publication* 19. II. 1935.—Ed.)

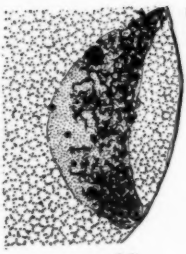

1
2
3
4
8
A
B
9
A
B
10
13
14
15
16
17



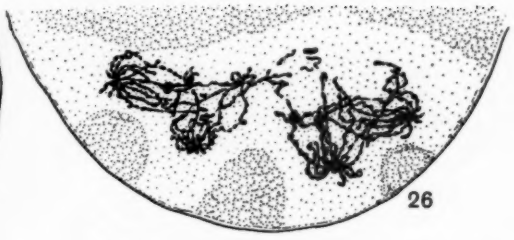
UNIV. OF MICH.



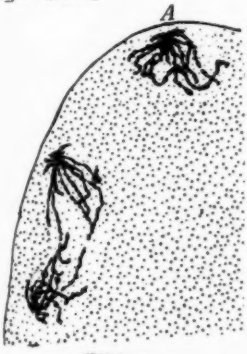
24



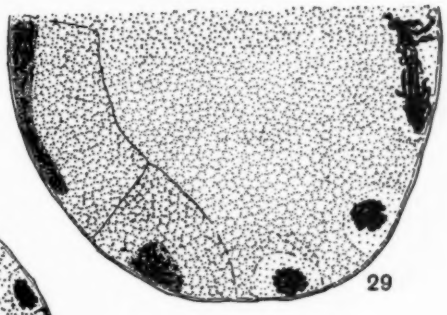
25



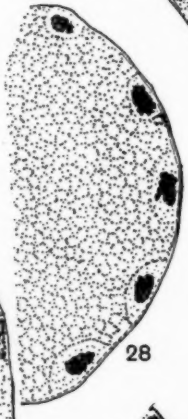
26



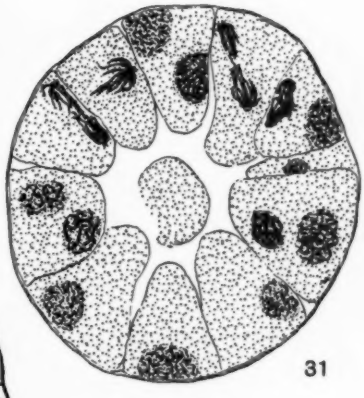
27



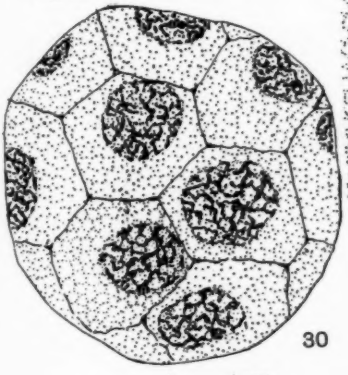
29



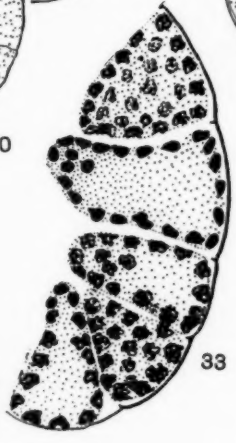
28



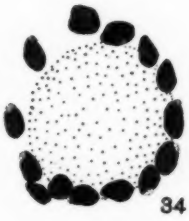
31



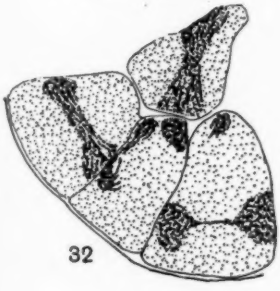
30



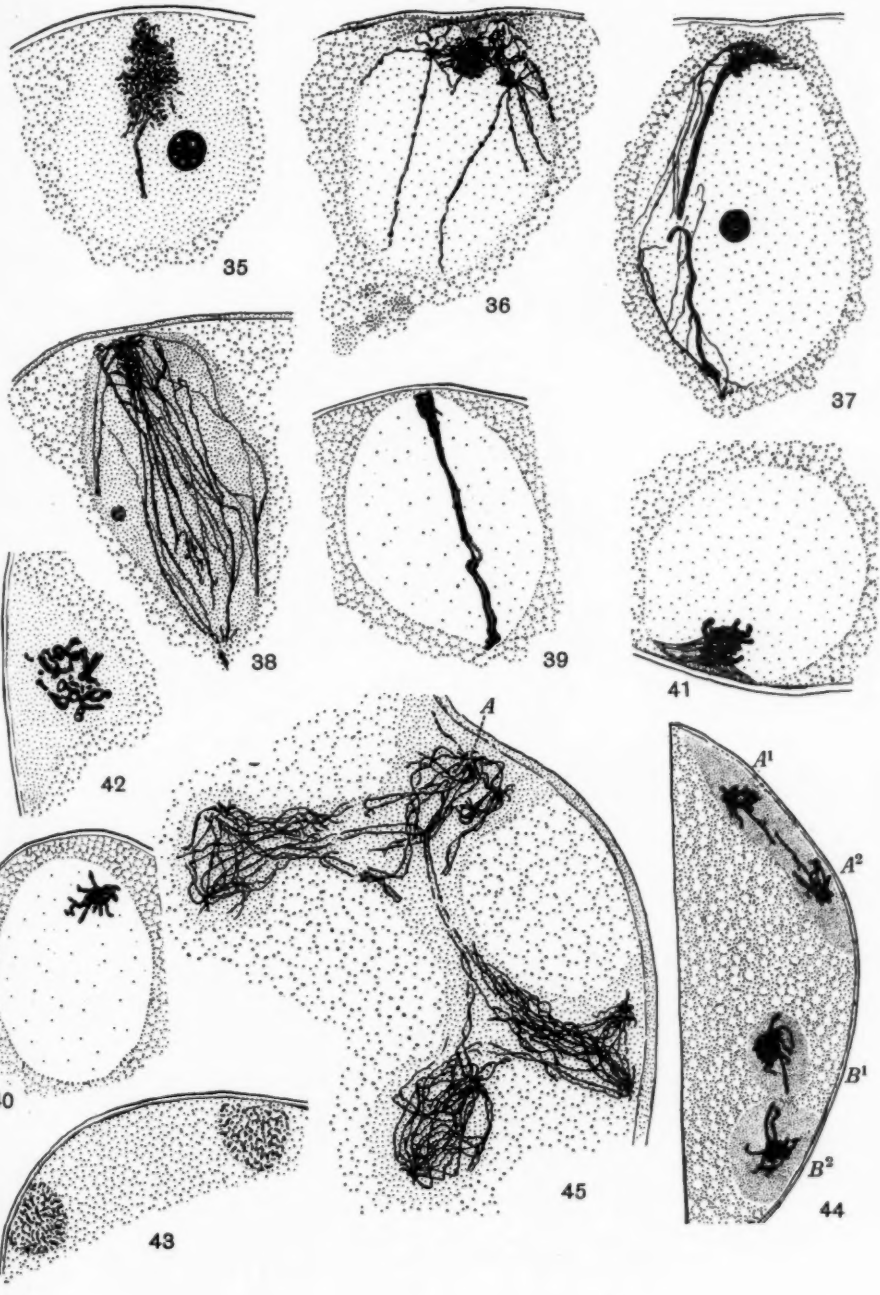
33



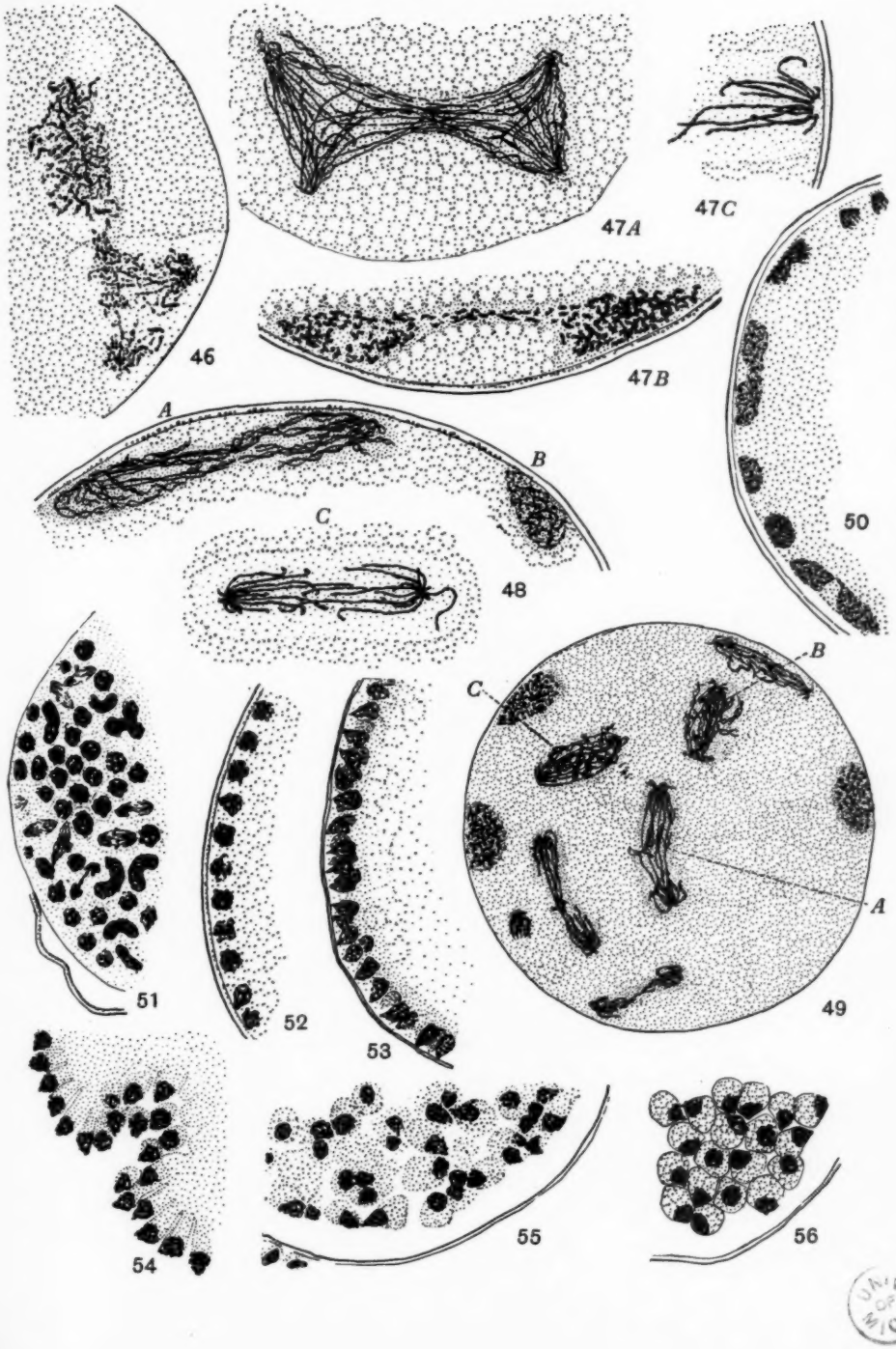
34



32



UNIV. OF MICH.



UNIV. OF MICH.

THE STRUCTURE AND TAXONOMIC POSITION OF *MEGASOLENA ESTRIX* LINTON 1910 (TREMATODA) WITH NOTES ON RELATED TREMATODES[1]

By H. W. MANTER

Zoological Laboratories, the University of Nebraska

(With 7 Figures in the Text)

I. Introduction

Linton (1910, pp. 38–9) described *Megasolena estrix* from *Kyphosus sectatrix* (Linn.), the Bermuda chub, at Tortugas, Florida. From Linton's description the trematode would seem to be a member of the Allocreadiidae in which family it has been placed by Poche (1925) and by Fuhrmann (1928). A study of seven specimens of this remarkable trematode shows Linton's description, based on a single specimen, to be inadequate for the proper taxonomic placing of the trematode. *Megasolena estrix* cannot be included in the family Allocreadiidae. It shows considerable resemblance to amphistomes and possesses many unusual distome characters.

The material on which this study is based was collected by the writer at the Biological Laboratory of the Carnegie Institution of Washington at Tortugas, Florida, during the summers of 1930, 1931 and 1932. For identification of the fish hosts, I am indebted to Dr W. H. Longley. Four specimens of *Megasolena estrix* were collected from the intestine of two of ten specimens of *Kyphosus sectatrix* (Linn.) and three specimens from a single *Kyphosus incisor* (Cuv. and Val.).

II. Description of *Megasolena estrix*

The body is smooth, elongate, cylindrical, more or less pointed posteriorly, widest at about the middle, 2·67–3·6 by 0·78–0·98 mm. The cuticula is very thick. Deep beneath the dorsal surface on each side immediately posterior to the oral sucker occurs a cluster of black pigment granules. These "eye spots" are rather deeply embedded in the parenchyma. The oral sucker is large, subterminal, with a diameter of 0·307–0·380 mm. The acetabulum, measuring 0·262–0·280 mm. in diameter, is ventral in position, 1/3–1/4 body length from the anterior end.

The mouth is ventral and subterminal. The oral sucker is followed by a distinct prepharynx, the anterior 1/2–2/3 of which is surrounded by a very thick layer of circular muscles. This conspicuous bulb of circular muscles is distinct from the oral sucker but sometimes overlaps (cup-like) the posterior

[1] Studies from the Zoological Laboratories, the University of Nebraska, No. 180.

end of the sucker. No true out-pocketings of the oral sucker occur, although an appearance of such sometimes is given when the posterior end of the sucker is enclosed by the muscular bulb. The pharynx is very large, usually but not always longer than wide, width 0·300–0·380 mm., length 0·307–0·380 mm. The long oesophagus extends to the posterior border of the acetabulum, where it forks to form the caeca which extend posteriorly to within a short distance of the posterior end of the body.

The body wall is composed of a thick cuticula which contains minute black granules of varying size and unknown significance; a distinct layer of circular muscles; a well-developed layer of longitudinal muscles, and a layer of oblique muscles. Small body muscles pass between the longitudinal muscles.

The genital pore, surrounded by conspicuous radial muscles, is ventral, well anterior to the acetabulum, near or at the level of the posterior end of the pharynx.

The two testes are median, tandem, close together, intercaecal, post-ovarian. The anterior testis, immediately posterior to the ovary is near mid-body region. The testes tend to be somewhat longer than wide, particularly the posterior testis. Their surfaces are smooth except for very slight waviness of outline. The vasa efferentia unite at the anterior border of the ovary and the vas deferens soon enlarges to form a broad, little coiled, tubular, external seminal vesicle which enters the posterior end of a large conspicuous hermaphroditic sac.

The ovary is smooth, transversely ovoid, median, immediately anterior to the testes, smaller than testes. The oviduct arises from near the anterior border of the ovary. A well-developed Laurer's canal, after coiling slightly, extends dorsally to open to the outside slightly posterior to the middle of the ovary. Mehlis' gland, lying anterior to the ovary is approximately the same size as the ovary. A seminal receptacle is lacking. The basal portion of the uterus is filled with sperm cells. The vitelline follicles fill the sides of the body from immediately posterior to the intestinal bifurcation to near the posterior end. They surround the caeca and are confluent posterior to the testes. The yolk

---

Explanation of Figs. 1–7.

Figures were drawn with the aid of a camera lucida except Figs. 2, 3 and 4 which are diagrammatic. The value of the enlarged scale is indicated in mm. Abbreviations: *es*, esophagus; *et*, excretory tubule; *ex*, excretory vesicle; *ga*, genital atrium; *gs*, genital sinus; *hs*, hermaphroditic sac; *lm*, lymph vessel; *mg*, Mehlis' gland; *mt*, metraterm; *pb*, prepharynx bulb; *sve*, external seminal vesicle; *svi*, internal seminal vesicle; *ut*, uterus.

Fig. 1. *Megasolena estrix*. Dorsal view.

Fig. 2. Diagram of *M. estrix* to show the four longitudinal lymph vessels.

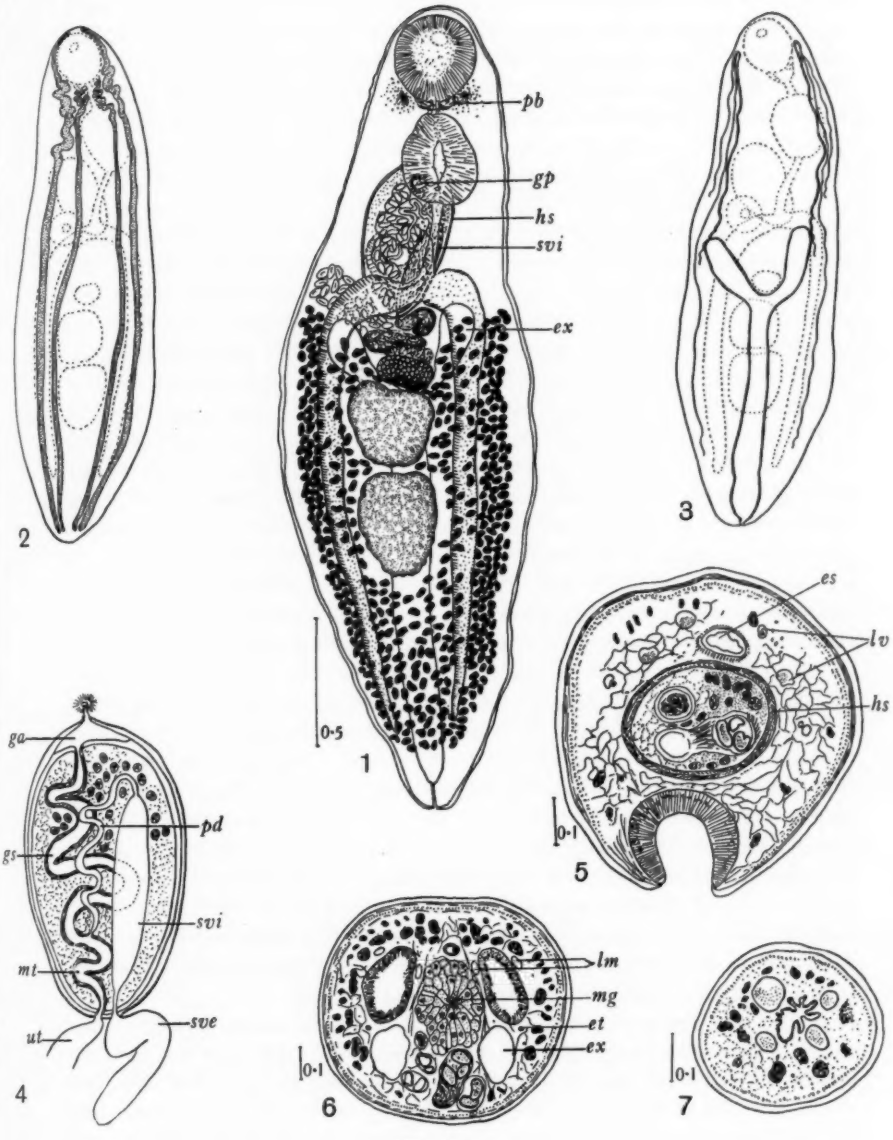
Fig. 3. Diagram of *M. estrix* to show the excretory vesicle and tubules.

Fig. 4. Diagram of the hermaphroditic sac of *M. estrix*.

Fig. 5. Cross-section of *M. estrix* at the level of the acetabulum.

Fig. 6. Cross-section of *M. estrix* at the level of Mehlis' gland.

Fig. 7. Cross-section of *M. estrix* near the posterior end, showing the median excretory vesicle and the four lymph vessels.


pb
gp
hs
svi
ex
0·5
1
2
3
es
lv
hs
0·1
5
ga
pd
gs
svi
mt
sve
ut
4
lm
mg
et
ex
0·1
6
0·1
7

Figs. 1–7.

reservoir is dorsal to the posterior half of the ovary. The uterus is short, preovarian and extends, on either the right or left side, lateral to the caecum, anterior to the vitellaria, to near the edge of the body. It then bends medianly to enter the posterior end of the hermaphroditic sac. The eggs are yellow, with fairly thin shells, and measure 60–76 by 30–34 μ.

The hermaphroditic sac (Fig. 4) is a characteristic organ. It is a large ovoid sac extending from midpharynx to the intestinal bifurcation. Rounded at both ends, it is slightly more narrow posteriorly. Its thick muscular wall is composed of an inner layer of diagonal muscles and an outer layer (of equal thickness) which consists of diagonal fibres posteriorly but of more circular muscles in the anterior half of the sac. The internal seminal vesicle is a large, uncoiled, almost straight tube extending to near the anterior end of the sac. Here it is continuous with a fine pars prostatica or prostatic duct which coils posteriorly and joins the thick-walled uterus or metraterm two-thirds the length of the sac from the anterior end. From this point forward the metraterm, enclosed by the muscular, hermaphroditic sac, although its morphological character has not changed, must be known as a genital sinus. It opens into a fairly spacious genital atrium distinctly separated from the remainder of the hermaphroditic sac but walled by an extension of the outer muscular layer of the sac. A short tube leads ventrally from the atrium to the genital pore. Outside the wall of the atrium this tube is surrounded by radial muscles which do not form, however, a true genital sucker since they have no outer membrane but end in the parenchyma. There is no genital cone or papilla. In addition to the coiled, thick-walled metraterm and the male ducts, the hermaphroditic sac contains parenchyma-like cells and gland cells.

The excretory pore is at the posterior end of the body. The excretory vesicle is Y-shaped with long median stem dorsal to the testes forking at the posterior end of the ovary to form two short wide crura which extend anteriorly (ventral to the caeca) to the intestinal bifurcation. From near the tip of each crus arises a collecting tubule which, after sending a very short branch posteriorly, extends anteriorly to the oral sucker where it bends posteriorly and branches into two tubules (Fig. 3). One of these tubules ends at the level of the intestinal bifurcation, but the other extends backward to within a short distance of the posterior tip of the intestinal caecum on each side. Cross-sections, then, anterior to the intestinal bifurcation show three excretory tubules on each side, one ascending, two descending. Cross-sections posterior to the intestinal bifurcation show one tubule (Fig. 6).

Four, well-developed, longitudinal lymph vessels occur, extending the entire length of the body (Fig. 2). Originating separately near the posterior end of the body, one pair extends anteriorly median to the caeca, the other pair lies dorsal to the caeca. Only four vessels can be seen in cross-sections of the body. In the region of the prepharynx in one specimen there seemed to be connecting vessels, an appearance probably due to the swollen, convoluted form of the vessels in this region. Conditions in two other specimens

sectioned indicate that the size of the vessels varies at different levels where evidently the tubes become swollen or collapsed according to the volume of lymph. Two vessels extend to the extreme anterior tip of the body, curving around the anterior border of the oral sucker and ending blindly. The other pair seems to end near the posterior end of the oral sucker. The vessels tend to remain close to the intestinal caeca, show no connection with the acetabulum, and are not connected with the excretory system. A finely granular material fills these lymph vessels.

III. The genus *Hapladena* Linton

Linton named the genus *Hapladena* with its type species *varia* in 1910 from *Teuthis hepatus* and *Teuthis coeruleus* (now known as *Acanthurus hepatus* and *A. coeruleus*) at Tortugas, Florida. Linton did not indicate the family connections of this species and it has been listed as an unclassified genus by Poche (1925) and Fuhrmann (1928).

This species has been collected at Tortugas by the author from *Acanthurus hepatus* (Linn.), *A. coeruleus* Bloch and Schneider, *Sparisoma flavescens* (Bloch and Schneider) and *Pseudoscarus plumbaeus* Bean. A complete description of *Hapladena varia* will be given in a later paper. *Hapladena* like *Megasolena* possesses four conspicuous, longitudinal lymph vessels in approximately the same position. *Hapladena* possesses not a cirrus sac but an hermaphroditic sac fundamentally similar to that of *Megasolena*. In *Hapladena*, this sac is more elongate and less muscular but contains a muscular metraterm, long genital sinus, seminal vesicle, prostatic duct and gland cells. The arrangement of these parts is the same in both genera. In *Hapladena*, the prostatic duct joins the metraterm at about mid-sac level. Peculiar spine-like but non-chitinous structures occur in the basal region of the genital sinus near the level of the entrance of the prostatic duct. In *Halpladena*, a special group of muscles at the anterior end of the pharynx suggests the prepharynx bulb of *Megasolena*. The excretory system of *Hapladena* is like that of *Megasolena* except that the arms or crura of the vesicle are smaller, shorter and extend almost laterally. *Hapladena* also has a far anterior, well-developed acetabulum. *Hapladena* differs from *Megasolena* in possessing one testis, a seminal receptacle, smaller pharynx, body spines and in lacking eye spots and radial muscles around the genital pore. The body spines of *Hapladena* seemed entirely lacking in nine of eleven specimens and must be easily lost even before death.

IV. Discussion.

It is evident that neither *Megasolena* nor *Hapladena* can be classified in the Allocreadiidae. The character of the excretory system, the lymph vessels and the peculiar hermaphroditic sac are entirely unlike the Allocreadiidae.

The importance of the lymph system in trematodes was recognised first by Looss in 1912. Its occurrence and significance has been discussed recently by Stunkard (1929) and by Willey (1930, 1933). This system of vessels is well

known among the amphistomes and in the Angiodictyidae (a family of monostomes). "Definite lymph channels" have been noted in the Cyclocoelidae and the Heronimidae (also monostomes). Willey (1930) suggests that amphistomes of warm-blooded vertebrates may be found to possess a single pair of lymph vessels (as is known in *Cotylophoron* Stiles and Goldberger, mammalian species of *Paramphistomum* Fischoeder, *Stephanopharynx* Fischoeder, *Chiorchis* Fischoeder, and *Zygocotyle lunatum* (Dies.)), whereas amphistomes of cold-blooded hosts may be found to possess three pairs of vessels (as is known for *Schizamphistomoides spinulosum* (Looss), *Schizamphistomum scleroporum* (Crep.), *Allassostoma* Stunkard and *Paramphistomum stunkardi* Holl). *Diplodiscus temporatus* Stafford has four pairs of vessels arising, however, from a fundamental three-pair condition. Bhalerao (1931) described two pairs of lymph vessels for *Stunkardia dilymphosa* Bhalerao, an amphistome from *Batagur baska*, an Indian tortoise. So far as I can learn, no definite system of lymph vessels has been described for a distome. Stunkard (1928) mentions a lymphatic vesicle in *Vasotrema amydae* Stunkard, a member of the Spirorchidae. Alvey and Martin (1934) report two lymph vessels in *Sphyranura oligorchis* Alvey, a monogenetic trematode of *Necturus*.

The lymph system in *Megasolena* and *Hapladena* most resembles that found in *Stunkardia* Bhalerao, but this latter genus is entirely different in other respects. The excretory system of *Megasolena* is different from that of most amphistomes or of Angiodictyidae but is similar to that of *Gyliauchen papillatum* (Goto and Matsudaira). The hermaphroditic sac of *Megasolena* is fully as unique as the lymph system. In some distomes (*e.g.* Hemiuridae) the ductus hermaphroditicus is surrounded by a sac which has been called a sinus sac (Manter, 1926, p. 92). Such a structure, however, is entirely different from the cirrus-sac-like structure found in *Megasolena*. Here, the sac encloses the coiled metraterm as well as the internal seminal vesicle, pars prostatica, gland cells, ductus hermaphroditicus and genital atrium. It is more than a sinus sac. I know of no such structure except in *Brumptia gigas* (MacCallum), an amphistome of the African elephant. Fukui (1929) recognised it as characteristic of the subfamily Brumptinae and suggested (p. 269) the appropriate name "hermaphroditic pouch". The similarity of this sac in *Megasolena* to that in *Brumptia* is remarkable. Maplestone (1923, p. 199), who refers to it as a cirrus sac, describes the internal seminal vesicle, the pars prostatica "curving posteriorly until it ends by uniting with the uterus near the centre of the pouch", and the "loose areolar tissue" within the sac. The lymph vessels of *Brumptia* seem to be unknown but this amphistome is markedly different from *Megasolena* except in the agreement in the structure of the hermaphroditic pouch.

The peculiar prepharynx bulb of *Megasolena* is not entirely unique. *Maculifer* Nicoll 1915 possesses apparently the same structure. Like the other most peculiar characters of *Megasolena*, this structure can be traced to amphistomes. An undescribed trematode collected from *Diodon holocanthus* Linn.

at Tortugas by the writer is considered a second species of *Opistholebes* Nicoll 1915. *Opistholebes* is an amphistome from *Sphaeroides lunaris* in Australia. This fish is related to *Diodon*. *Opistholebes* has many characters differing from most amphistomes and has been classified by Fukui (1929) in a distinct family with *Gyliauchen* Nicoll 1915. The American species is very similar to the Australian except that it possesses a well-developed prepharynx bulb exactly as in *Megasolena* and *Maculifer*. Furthermore, the American species shows scattered, heavy pigment flecks as does *Maculifer*. *Maculifer*, like *Megasolena*, has been listed in the Allocreadiidae, but it now seems very possible that it will be found to possess a lymph system.

Opistholebes does not possess an hermaphroditic sac. Its lymph vessels although present (in my species, at least) are too inconspicuous to be traced in the specimen studied. There seem to be three pairs.

Megasolena and *Hapladena*, therefore, exhibit a peculiar and remarkable set of characters for counterpart of which we must go to trematodes of an African elephant, an Indian tortoise and Australian and Asiatic fish. The picture will doubtless show more harmony when the large gaps in our knowledge of trematodes of marine fish are filled in.

V. TAXONOMIC POSITION OF *MEGASOLENA* AND *HAPLADENA*

The amphistome affinities of *Megasolena* and *Hapladena* are seen in the lymph vessels, hermaphroditic sac, cylindrical body, large Mehlis' gland and in other minor details, and are supported by the fact that the prepharynx bulb of *Megasolena* can be traced to amphistomes (*Opistholebes*). This prepharynx bulb is also suggested in some Angiodictyidae (*e.g. Octangium* and especially *Octangium takanoi* Kobayashi). *Megasolena* and *Hapladena* differ from all amphistomes and from Angiodictyidae in the anterior position of the acetabulum. The pretesticular position of the ovary and the excretory system are unlike most amphistomes but like the condition found in *Gyliauchen* and *Opistholebes*. Although the hermaphroditic sac resembles that of *Brumptia* and is a character considered of subfamily rank by Fukui and although the lymph system resembles that of *Stunkardia*, the closest relatives of *Megasolena* and *Hapladena* are probably *Opistholebes*, *Maculifer* and *Gyliauchen*. *Opistholebes* and *Gyliauchen* are known to have a lymph system and all three genera possess the muscular bulb around the prepharynx. For the present, it is suggested that these genera be grouped in a single family. Fukui has already considered *Opistholebes* and *Gyliauchen* in the same family and Goto (1919, p. 46) shrewdly observed that "*Opistholebes* appears to be related to Nicoll's *Maculifer subaequiporus*".

The taxonomy of this family is somewhat confused. *Dissotrema* Goto and Matsudaira 1918 was named type of the family Dissotrematidae Goto and Matsudaira 1918. The following year Goto (1919) reduced the genus *Dissotrema* to a synonym of *Gyliauchen* Nicoll 1915 but did not mention a family name. Poche (1925) and Fuhrmann (1928) consider *Dissotrema* and *Gyliauchen* dis-

tinct and classify them in the family Dissotrematidae. Fukui (1929) considers *Gyliauchen* (and its synonym *Dissotrema*) in the same family with *Opistholebes* (hitherto unclassified) and names a new family, Opistholebetidae. Ozaki (1933) refers *Gyliauchen* to the family Gyliauchenidae (Goto and Matsudaira). Yamaguti (1934) includes *Gyliauchen* in the Opistholebetidae Fukui 1929. Travassos (1934) considers *Opistholebes* and *Gyliauchen* in different families and recognizes Opistholebetidae Fukui 1929 and Gyliauchenidae Ozaki 1933.

I agree with Goto, Ozaki and Yamaguti in considering *Dissotrema* a synonym of *Gyliauchen* and feel the genus can be included in the same family with *Opistholebes*. Although Fukui perhaps should have retained *Gyliauchen* as the type of this family, I cannot find that any rule was violated in the establishment of the Opistholebetidae. A description of the family modified to include *Megasolena* and *Hapladena* follows:

Family OPISTHOLEBETIDAE Fukui 1929.

Synonyms: Dissotrematidae Goto and Matsudaira 1918.

Gyliauchenidae Ozaki 1933.

Digenea with cylindrical or ovoid body. Oral sucker without evaginations. Acetabulum near posterior end or anterior to midbody. Prepharynx bulb usually present. Pharynx present. Caeca reaching to posterior end or only to midbody. Possessing one testis or two testes symmetrical, oblique or tandem. Cirrus sac or hermaphroditic sac present. Ovary pretesticular. Vitellaria lateral or anterior. Seminal receptacle present or lacking. Excretory system Y-shaped with a recurrent branch on each side. Lymph system present. Parasites of fish. Type genus: *Gyliauchen* Nicoll 1915.

Subfamily GYLIAUCHENINAE Fukui 1929.

Subfamily OPISTHOLEBETINAE Fukui 1929.

Subfamily MEGASOLENINAE n. subfamily.

VI. DIAGNOSIS OF MEGASOLENINAE N. SUBFAMILY

Body smooth or with evanescent spines. Oral sucker large. Acetabulum well developed, anterior to midbody. A special group of muscles associated with the prepharynx. Pharynx present. Ovary pretesticular. Vitellaria lateral. Seminal receptacle present or lacking. Laurer's canal present. Large hermaphroditic sac containing internal seminal vesicle, pars prostatica, gland cells, parenchyma-like tissue, metraterm, genital sinus. Excretory vesicle Y-shaped with recurrent tubule on each side. Two pairs of lymph vessels. Intestinal parasites of marine fish. Type genus *Megasolena* Linton 1910. Other genus *Hapladena* Linton 1910. *Maculifer* will probably be found to belong in this subfamily.

VII. SUMMARY

1. *Megasolena estrix* Linton 1910 has been incorrectly classified in the Allocreadiidae.

2. The possession of two pairs of longitudinal lymph vessels, a remarkable

hermaphroditic sac as well as other minor characters indicate relationship to the Paramphistomidae.

3. *Hapladena* Linton 1910 possesses these same characters and is considered related to *Megasolena*.

4. These characters are considered to be of more fundamental taxonomic value than the position of the acetabulum.

5. A new subfamily, Megasoleninae, of the family Opistholebetidae is named and described.

REFERENCES

ALVEY, C. H. and MARTIN, W. E. (1934). The lymph system of *Sphyranura oligorchis* (abstract). *J. Parasit.* **20**, 327.

BHALERAO, G. D. (1931). Two new trematodes from reptiles: *Paryphostomum indicum* n.sp. and *Stunkardia dilymphosa* n.g., n.sp. *Parasitology*, **23**, 99–108.

FUKUI, T. (1929). Studies on Japanese amphistomatous parasites, with revision of the group. *Jap. J. Zool.* **2**, 219–351.

FUHRMANN, O. (1928). Trematodes in Krumbach's *Handbuch der Zoologie*, **2**, Lief. 3.

GOTO, S. (1919). *Dissotrema* synonymous with *Gyliauchen*. *J. Parasit.* **6**, 44–7.

GOTO, S. and MATSUDAIRA, Y. (1918). On *Dissotrema papillatum* n.g., n.sp., an amphistomoid parasite from a marine fish. *J. Coll. Sci. Tokyo*, **39**, 1–19.

LINTON, EDWIN (1910). Helminth fauna of the Dry Tortugas. II. Trematodes. *Publ. Carneg. Inst. Wash.* No. 133, 11–98.

LOOSS, A. (1912). Über den Bau einiger anscheinend seltener Trematoden Arten. *Zool. J.* Suppl. **15**, 323–66.

MANTER, H. W. (1926). Some North American fish trematodes. *Illinois biol. Monogr.* **10**, 1–138.

MAPLESTONE, P. A. (1923). A revision of the Amphistomata of mammals. *Ann. trop. Med. Parasit.* **17**, 113–212.

NICOLL, WM. (1915). The trematode parasites of North Queensland. III. Parasites of fishes. *Parasitology*, **8**, 22–40.

OZAKI, Y. (1933). *Telotrema caudatum* n.g., n.sp., ein neuer Typus der Trematoden-familie Gyliauchenidae (Goto et Matsudaira). *Zool. Anz.* **103**, 329–32.

POCHE, F. (1925). Das System der Platodaria. *Arch. Naturgesch.* **91** A, 1–458.

STUNKARD, H. W. (1928). Observations nouvelles sur les Trématodes sanguicoles du genre *Vasotrema* (Spirorchidae) avec description des deux espèces nouvelles. *Ann. Parasit.* **6**, 303–20.

—— (1929). The parasitic worms collected by the American Museum of Natural History Expedition to the Belgian Congo, 1909–14. *Bull. Amer. Mus. nat. Hist.* **58**, 233–89.

TRAVASSOS, LAURO (1934). Synopse das Paramphistomoidea. *Mem. Inst. Oswaldo Cruz*, **29**, 19–178.

WILLEY, C. H. (1930). Studies on the lymph system of digenetic trematodes. *J. Morph. Physiol.* **50**, 1–38.

—— (1933). The lymph system of *Zygocotyle lunatum* (Trematoda, Paramphistomidae). *Parasitology*, **25**, 242–7.

YAMAGUTI, S. (1934). Studies on the helminth fauna of Japan. Part 2. Trematodes of fishes, I. *Jap. J. Zool.* **5**, 249–541.

(*MS. received for publication* 22. II. 1935.—Ed.)

TWO NEW FUNGI OF THE GENUS *COELOMOMYCES* PARASITIC IN LARVAE OF *ANOPHELES*

By M. O. T. IYENGAR

From the London School of Hygiene and Tropical Medicine

(With 5 Figures in the Text)

In India larvae of several species of *Anopheles* (Culicidae, Nematocera, Diptera) are parasitised by two species of fungi which form numerous large-sized spores in the host. These fungi occur in the haemocele of the *Anopheles* larva, and in heavily infected larvae the fungus can be seen to fill the entire haemocele from the thorax to the last segment of the abdomen. In many instances mycelia and spores occur even within the head of the insect. The fungi were not observed to invade the anal gills of the larva (except in a solitary instance in which spores occurred within the deteriorating anal gills).

The spore-like bodies observed in these parasitised larvae are the sporangia[1] of two new species of parasitic fungi allied to known species of the genus *Coelomomyces* Keilin, *C. stegomyiae* Keilin 1921 and *C. notonectae* (Bogayavlensky) 1922, parasitic on *Aedes* (*Stegomyia*) *albopictus* Skuse (Culicidae, Diptera) and *Notonecta* sp. (Heteroptera, Rhynchota), respectively. The two new species observed parasitic in larvae of *Anopheles* in India are named *Coelomomyces indiana* sp.nov., and *C. anophelesica* sp.nov., respectively. These, although closely allied to the two known species of *Coelomomyces*, present some outstanding differences.

Larvae of the following species of *Anopheles* have been observed infected with these fungi: *A.* (*Anopheles*) *barbirostris* v. d. Wulp, *A.* (*A.*) *hyrcanus* var. *nigerrimus* Giles, *A.* (*Myzomyia*) *subpictus* Grassi, *A.* (*M.*) *vagus* Dön., *A.* (*M.*) *annularis* v. d. Wulp, *A.* (*M.*) *jamesi* Theo., *A.* (*M.*) *ramsayi* Covell, *A.* (*M.*) *varuna* Iyengar, and *A.* (*M.*) *aconitus* Dön.

The following general observations on the infection refer to both species of *Coelomomyces* described in this article.

The development of the parasite is completed during the larval phase of the mosquito. The infection probably sets in during the first instar of the larva; fully developed sporangia are found in the third and fourth larval instars. The progress of the infection is therefore remarkably rapid. Con-

[1] In this article, the spore-like bodies observed in these infections are termed "sporangia" in view of Keilin's (1921) description and figures with reference to *Coelomomyces stegomyiae*. It should be noted that the further development of these bodies is not known, and it is now difficult to say with any certainty whether they are true spores or sporangia. Following, however, the terminology used by Keilin, the writer speaks of these spore-like bodies as sporangia without making any assumption as to the further development of these structures.

sidering that the larval phase of *Anopheles* in the tropics does not ordinarily exceed 10 days, the time taken for the parasite to grow in the host from the onset of the infection to the formation of fully developed sporangia would be much shorter than the larval phase of the mosquito and would be approximately 6 days.

The infection generally starts in the thoracic region and spreads posteriorly into the abdominal segments travelling along the adipose tissue on which the fungus lives. The fat-body as a result of the attack by the fungus loses its characteristic appearance and shrinks, the nuclei disintegrate and

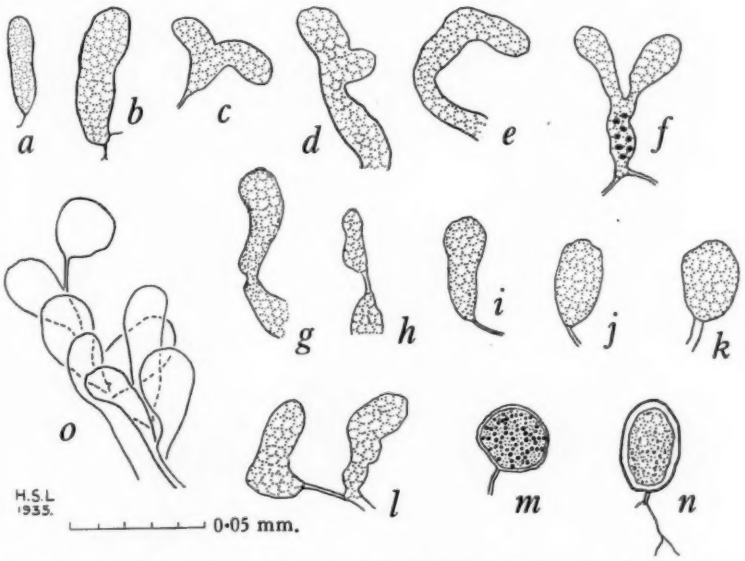


Fig. 1. *Coelomomyces indiana* sp.nov. *a* and *b*, young mycelia with hyphae at base; *c–f*, older mycelia; *g*, *h* and *l*, formation of the sporangium by constriction; *i*, *j*, *k*, *m* and *n*, development of the sporangium; *o*, a group of mycelia forming sporangia. (Camera lucida, H.S.I. *del.*)

finally the fat-body disappears entirely. In place of the fat-body, a thin membrane is left which is filled with numerous dark brown pigment granules. A parasitised larva in the later stages of the infection has practically no fat tissue. It is only rarely that an infected larva of the fourth instar has any fat tissue, and the presence of fat in such a larva would indicate that the infection has been of a low intensity.

Another effect of the infection is the suppression of the imaginal buds. While in the normal larva of the fourth instar the wing and leg rudiments are well developed, in the parasitised larva of the same instar these structures are not fully developed. It seems improbable therefore that an infected larva would ever complete its development into the adult mosquito. In a few

exceptional cases, the infected larva pupated and the adult emerged, but such instances are rare. Ordinarily, infected larvae die before pupating.

The fungus can be seen in dissections of infected larvae or in serial sections. Two phases of the parasite can be seen: (1) the mycelial phase which is tubular, and (2) the sporangial phase consisting, in the early stages, of oval or round transparent bodies and in the later stages, of opaque yellow bodies with a thick wall and definite sculpturing of the wall. In the early stages of the infection, the tubular mycelial form constitutes the bulk of the parasite in the host, while in the later stages the sporangium is the predominant form.

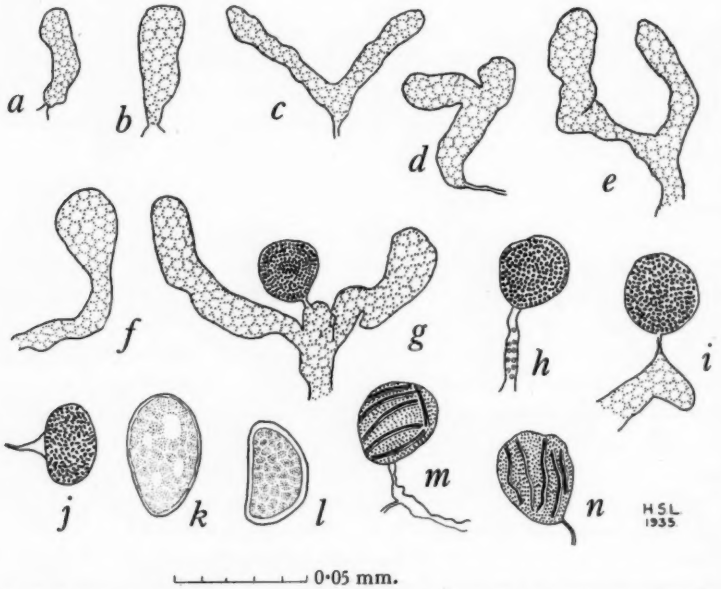


Fig. 2. *Coelomomyces anophelesica* sp.nov. *a* and *b*, young mycelia; *c–f*, older mycelia; *g* and *i*, formation of sporangium; *h*, formation of sporangial wall and withdrawing of contents of mycelium; *j*, young sporangium with attached strand; *k*, vacuolated stage of sporangium; *l*, older sporangium with dense contents and thick wall; *m* and *n*, mature sporangia with partially developed ribs and connecting strand. (Camera lucida, H. S. L. *del.*)

The mycelium is thin-walled, tubular and attached to the fat-body. It either protrudes out from the fat-body or runs alongside closely adpressed to it. In dissections of infected larvae very minute and short hyphae measuring about $\frac{1}{2}\mu$ thick are often recognised at the base of the mycelium, and they appear to penetrate the fat tissue and serve to fix the mycelium to the fat-body. The mycelia are short and give rise to short branches which are of the same thickness as the basal mycelium. They do not form anastomoses and in this respect differ from *Coelomomyces stegomyiae* and *C. notonectae* which form definite anastomoses.

The mycelium has no cross-walls and is unicellular; it is filled with dense protoplasm and has a large number of nuclei. Apically the mycelium is often swollen and sporangia arise by abstriction near the apices of the mycelia. A short distance below the apex, the mycelium constricts and its contents separate at the constriction leaving a clear area devoid of protoplasm

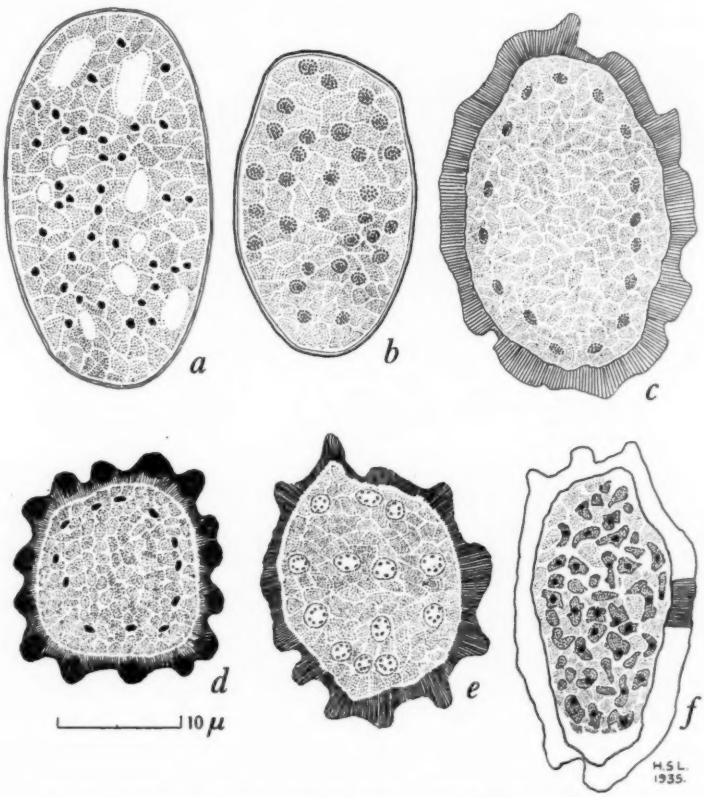


Fig. 3. *Coelomomyces indiana*: sections of sporangia in different stages of development. *a*, longitudinal section of vacuolated sporangium; *b*, sporangium with dense contents and thicker wall; *c*, longitudinal section through mature sporangium and, *d*, transverse section showing peripheral distribution of nuclei and structure of spore wall; *e*, later stage showing nuclei distributed all over contents; *f*, section of mature sporangium showing segmentation of contents. (Camera lucida, H. S. L. *del.*)

between the future sporangium and the mycelium. The contents above the constriction collect together to form a globular or oval mass, and the subsequent formation of a wall round it cuts off the sporangium from the mycelium. In the early stages the sporangium does not separate from the mycelium but remains attached to it by a thin transparent strand devoid of

protoplasm and measuring about 1 μ thick. This strand is the collapsed wall of the mycelium from which the contents have withdrawn. These strands are not easily recognised as they do not take stain. At a later stage the connecting strand may break due to pressure and the sporangium be liberated into the haemocele.

When differentiated from the mycelium, the sporangium is thin-walled and its contents are dense. Subsequently, the contents become vacuolated.

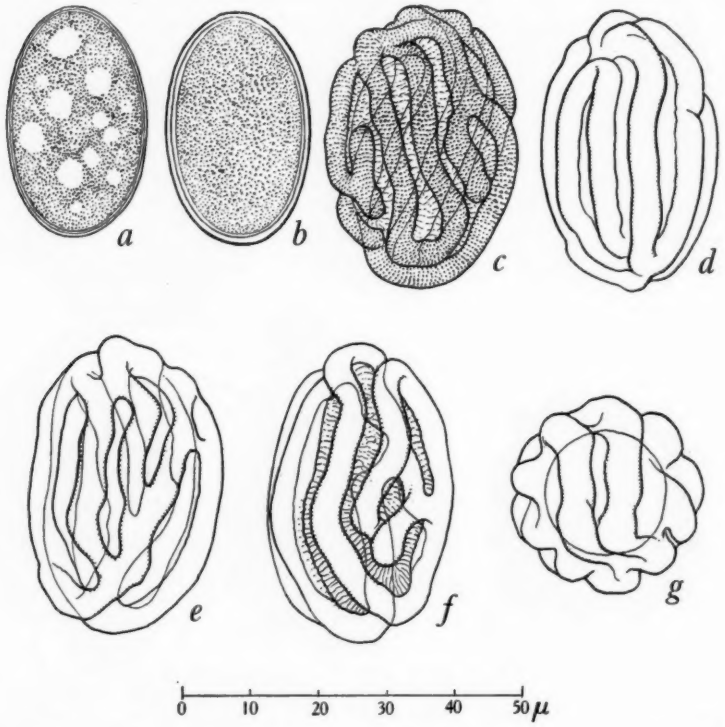


Fig. 4. *Coelomomyces indiana*: sporangia. *a*, vacuolated sporangium; *b*, sporangium with thicker wall and dense contents; *c–f*, mature sporangia with different patterns of sculpturing on wall; *g*, mature sporangium as seen from one end. (Camera lucida, E. Terzi *del.*)

In the next stage in its development, the contents of the sporangium condense and the sporangium becomes an oval body with a thick refractile wall. The vacuoles that existed in the previous stage disappear and the number of nuclei is much larger than in any of the prior stages. In *C. anophelesica*, the sporangium at this stage and in the previous stage shows a tendency to have one side flat and the other strongly convex.

In the subsequent stage, the wall of the sporangium forms external ridges

and turns yellow. The full-grown sporangium is ribbed and has a characteristic sculptural effect on the wall.

The mature sporangium has a decidedly smaller number of nuclei than the stage immediately preceding it. In the mature sporangium of *C. indiana*,

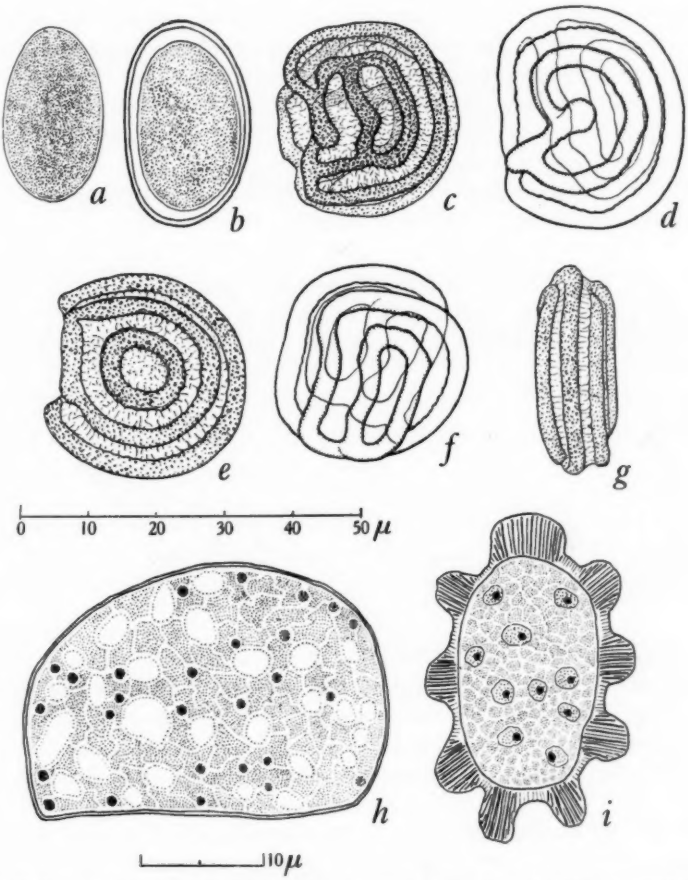


Fig. 5. *Coelomomyces anophelesica*: sporangia. *a*, young sporangium; *b*, older sporangium with thicker wall; *c–f*, mature sporangia showing different patterns of sculpturing on wall; *g*, mature sporangium as seen from edge; *h*, longitudinal section of sporangium of the vacuolated stage; *i*, transverse section of mature sporangium showing structure of spore wall and distribution of nuclei. (Camera lucida; *a–g*, E. Terzi *del.*, *h* and *i*, H. S. L. *del.*)

the nuclei are at first distributed at the periphery. Later the nuclei are scattered all over the contents of the sporangium. At a still later stage, there is an indication of the separation of the contents into numerous irregular masses, each probably with a nucleus. But beyond the suggestion of seg-

mentation of the contents, no advanced stages similar to what Keilin (1921) describes as formation of spores have been observed, although serial sections of a large number of parasitised larvae were examined.

In the mature sporangium of *C. anophelesica*, the only stage observed was the distribution of the nuclei all over the contents of the sporangium. Two stages observed in mature sporangia of *C. indiana*, namely, the peripheral distribution of the nuclei and the segmentation of the contents, were not observed in *C. anophelesica*.

The wall of the mature sporangium is not homogeneous in structure; it is made up of alternating yellow and brown striae which are easily seen in sections of the sporangium.

The further development of the sporangium and the mechanism of infection of the *Anopheles* larva are still unknown.

Genus **Coelomomyces** Keilin

Parasitology, **13**, 225, 1921. Type-species *C. stegomyiae* Keilin.
Syn. *Zografia* Bogayavlensky, *Arch. russes Protist.* **1**, 113, 1922. Type-species *Z. notonectae* Bogayav.
Synonym by Keilin (1927).

Mycelium tubular, unicellular, multinuclear, either long, much branched and anastomosing or short and non-anastomosing; sporangium formed by abstriction at apex of mycelium, unicellular, plurinuclear and thick-walled. The sporangial wall smooth or with ribs forming a sculptural effect externally.

Parasitic in the body cavity of insects or their larvae.

The two new species of *Coelomomyces* from India are described below.

Coelomomyces indiana sp.nov.

Mycelium unicellular, fragile, very thin-walled and filled with dense protoplasm and numerous nuclei; pear-shaped in early stages, later tubular. Branches short, never anastomosing. Thickness of mycelium 7–14 μ, sometimes more, especially at apex. Mycelium attached to fat-body apparently by very minute hyphae.

Early sporangium globular or oval, thin-walled and measuring 20×30–$28 \times 40\,\mu$. Vacuolated sporangium nearly same size, oval and thin-walled. Sporangium of the next stage with thick refractile wall, dense protoplasmic contents and numerous nuclei. Size 20×30–$28 \times 42\,\mu$, sometimes larger. In the mature sporangium, the wall is thick, opaque and yellow, and has thick ribs running lengthwise from one end of the sporangium to the other. Sometimes the ribs branch considerably to form a net-like sculpturing on the wall. The sculpturing on the sporangial wall varies to some extent. Some of the patterns of sculpturing of the sporangia are shown in Fig. 4, *c–f*. Size of sporangium 25×38–$36 \times 60\,\mu$. The sporangium is typically oval in shape. Wall of sporangium in section shows alternating yellow and brown striae. Thickness

of wall at the ribs 2·5–3 μ and between the ribs 1 μ. Inner wall of sporangium corresponding to that observed in *C. stegomyiae* absent.

C. indiana was observed to parasitise larvae of the following species of *Anopheles*: *A.* (*Anopheles*) *barbirostris* v. d. Wulp, *A.* (*A.*) *hyrcanus* var. *nigerrimus* Giles, *A.* (*Myzomyia*) *subpictus* Grassi, *A.* (*M.*) *aconitus* Dön., *A.* (*M.*) *varuna* Iyengar, *A.* (*M.*) *ramsayi* Covell, *A.* (*M.*) *annularis* v. d. Wulp, and *A.* (*M.*) *jamesi* Theo.

Recorded from the following localities: Bengal (India)—Sonarpur, Dum-Dum, Behala, areas round Calcutta (24-Perganas District); Krishnagar (Nadia District); and Meenglas (Jalpaiguri District).

Coelomomyces anophelesica sp.nov.

Closely allied to *C. indiana*. Mycelium similar, measuring 7–12 μ thick, short and branched, non-anastomosing and attached to fat-body of host. Mycelium like that of *C. indiana* is very fragile and extremely thin-walled. Formation of sporangium similar to *C. indiana*. Early sporangium with dense protoplasm, globular or oval, measuring 19 × 28–23 × 37 μ. Vacuolated sporangium 20 × 30–24 × 40 μ, oval, or with one side flat and the other side convex. The next stage of sporangium with a thick refractile wall and dense contents and large number of nuclei. Mature sporangium asymmetrical, circular in shape, with one side flat and the other side strongly convex; thickness of sporangium less than breadth. Size 28 × 34–40 × 44 μ. Thickness 16–23 μ. Wall of sporangium with many raised ribs running in concentric or eccentric circles, the middle ribs branched and irregular. Some of the common types of sculpturing observed in these sporangia are shown in Fig. 5, *c–f*. When viewed at the edge, the sporangium is somewhat oval with ribs running parallel to each other (Fig. 5, *g*).

Structure of sporangial wall similar to *C. indiana* and composed of alternating light and dark striae. Inner spore-wall of *C. stegomyiae* absent. Thickness of wall at the rib 2·6–3·4 μ, and between the ribs 1·3 μ.

C. anophelesica was observed to parasitise larvae of the following species of *Anopheles*: *A.* (*Myzomyia*) *subpictus* Grassi, *A.* (*M.*) *vagus* Dön., *A.* (*M.*) *annularis* v. d. Wulp, and *A.* (*M.*) *varuna* Iyengar.

Distribution: Bengal (India)—Sonarpur (24-Perganas District); Krishnagar (Nadia District); and Meenglas (Jalpaiguri District).

Differentiation of the four species of Coelomomyces

Table I shows the differences between the four species of *Coelomomyces* at present known.

As will be seen from the table, the four species of *Coelomomyces* fall into two well-defined groups, the first group comprising *C. stegomyiae* and *C. notonectae*, and the second group comprising *C. indiana* and *C. anophelesica*. There is considerable difference between these groups. The species comprising the second group have a non-anastomosing mycelium which is very fragile

and which has a very thin wall. In the two species comprising the first group, the mycelium branches considerably and anastomoses. The mycelium has a thick wall.

Another difference between the two groups is in regard to the size of the mycelium. In the first group the mycelium is very narrow while in the other it is broader. In the former the mycelium is either attached to the viscera or lies free in the body cavity. In *C. stegomyiae* the mycelium forms a dense ramification round the midgut and the caeca. In *C. notonectae*, "the mycelium is always found lying free between the lobes of the fat-body and never penetrates inside the cells of that tissue" (Keilin, 1927, p. 365). In the second group, the mycelium is always attached to the fat-body; sections show clearly

Table I

Mycelium	Thickness of mycelium	Sporangium	Shape of sporangium	Sporangial wall	Inner wall	Peripheral distribution of nuclei	Segmentation of contents of sporangium	Host
C. stegomyiae: Distinct wall present. Anastomosing. Attached to viscera	2–6 μ	20 × 37·5–30 × 57 μ	Oval	Smooth surface, pitted	Present	Not seen	Divides into small spores	Larva of *Aedes* (*Stegomyia albopictus*)
C. notonectae: Distinct wall present. Anastomosing. Not attached to fat-body	? Similar to *C. stegomyiae?*	? Similar to above	Oval	Smooth surface, not pitted	Absent	Present	Divides into numerous amoeboid masses	Adult *Notonecta*
C. indiana: Wall very thin and membranous. Not anastomosing. Attached to fat-body	7–14 μ	25 × 38–36 × 60 μ	Oval	Ribbed surface; striae in section	Absent	Present	Faint indication of segmentation into irregular masses	Larva of *Anopheles*
C. anophelesica: Wall very thin and membranous. Not anastomosing. Attached to fat-body	7–12 μ	28 × 34–40 × 44 μ	Flat and somewhat circular	Do.	Absent	Not seen	Not seen	Larva of *Anopheles*

the attachment of the mycelium to the fat-body, the mycelium being either adpressed to the fat-body or its base embedded in between the peripheral cells of the fat-body.

A further difference is in regard to the presence of ribs on the wall of the sporangium. In the first group the wall is smooth, while in the second group there is a definite sculpturing produced by well-developed ribs.

The essential difference, however, between the two groups is in regard to the character of the mycelium. In the two new species described in this article, the short and simply branched mycelium with extremely thin wall is a marked divergence from the already known species of *Coelomomyces*. Considering this important difference, the erection of a new genus for the reception of the two new species was considered. Our information about these fungi is, however, so meagre that it seems advisable to err on the side of lumping together what are obviously very closely allied forms than to lay stress on the differences.

Systematic position of the genus

Nothing so far has been observed that could give a clear idea of the systematic position of the genus *Coelomomyces*. It is undoubtedly not a Myxomycete of the type of *Sporomyxa* as suggested by Bogayavlensky (1922), since all the four species so far known have a definite mycelium. With regard to the two species *C. stegomyiae* and *C. notonectae*, Keilin thinks that they are closely related to the Chytridiales, at least in regard to the development of the sporangium; the type of mycelium observed in these two species is, however, uncommon in the Chytridiales. On the other hand, the type of mycelium observed in *C. indiana* and *C. anophelesica*, which is very thin-walled and non-anastomosing, is a common type found in Chytridiales, and it would be best at this stage to place this genus under the Chytridiales, although it may be that when further stages in the life history of these parasites are known, the position will have to be revised.

I am indebted to Mr J. Ramsbotham and Miss F. L. Stephens of the British Museum (Natural History) and to Prof. D. Keilin, F.R.S., for helpful suggestions and kind criticism in connection with this study. My thanks are also due to Dr W. W. Jameson and Prof. P. A. Buxton for facilities afforded to work at the London School of Hygiene and Tropical Medicine. I must also thank Mr H. S. Leeson and Mr E. Terzi for the drawings illustrating this article.

REFERENCES

BOGAYAVLENSKY, N. (1922). *Zografia notonectae* n.g., n.sp. *Arch. russes Protist.* **1**, 113–19.

KEILIN, D. (1921). On a new type of fungus: *Coelomomyces stegomyiae* n.g., n.sp., parasitic in the body-cavity of the larvae of *Stegomyia scutellaris* Walker (Diptera, Nematocera, Culicidae). *Parasitology*, **13**, 225–34.

—— (1927). On *Coelomomyces stegomyiae* and *Zografia notonectae*, fungi parasitic in insects. *Ibid.* **19**, 365.

(*MS. received for publication* 8. III. 1935.—Ed.)

TWO STRAINS OF STREAK: A VIRUS AFFECTING THE TOMATO PLANT

By KENNETH M. SMITH, D.Sc., Ph.D.

Potato Virus Research Station, School of Agriculture and Molteno Institute, Cambridge

(With Plates XV and XVI)

Introductory

There is increasing evidence that a plant virus as it occurs in nature is not always a pure strain but may consist of two or more closely similar strains which can be separated out by continuous subculturing.

There is also some evidence that a given strain may itself change or mutate during the process of subculturing (Jensen, 1933; Price, 1934). This occurrence of mixtures of strains must not be confused with the occurrence of mixtures of different *viruses* such as frequently happens in the potato plant (Murphy and M'Kay, 1932; Smith, 1931).

McKinney (1926, 1931) in his work with green and yellow mosaics in wheat and tobacco seems to have been one of the first to offer evidence on the existence of these strains. Salaman (1933) and the writer (Smith, 1933) have shown that closely similar strains occur in potato virus *X*, although at that date the writer was inclined to the view that the strains were not stable. More recently Jensen (1933) and Price (1934) have isolated green and yellow strains of tobacco virus 1 and cucumber mosaic respectively.

The present communication shows that the virus of tomato streak falls into line with these other viruses in this respect.

Experimental

The virus was obtained four years ago from a tomato plant sent in by a grower. The plant in question showed the typical symptoms of tomato streak disease (single virus streak) which used to be known as tomato stripe (Ainsworth *et al.* 1934). These symptoms consisted of a mottle on the leaves and gross necrotic lesions on the stem. Inoculations were made from this plant to various Solanaceous plants, and the symptoms produced in these will be shortly described.

Tobacco var. White Burley

The virus does not as a rule produce local lesions on the inoculated leaves, but when it does the lesions are followed by a severe necrosis which passes up the petiole to the main stem on which a gross lesion is formed (Pl. XV, fig. 1).

From this point the disease may take one of two forms, either the stem necrosis is followed by a complete wilting with subsequent death of the plant (Pl. XV, fig. 2) or else the plant makes a recovery from the necrotic phase and shows only a green mosaic mottle with slight distortion of the leaves.

Nicotiana glutinosa

Local lesions are invariably produced on the inoculated leaves. The subsequent progress of the disease in young plants closely resembles the necrotic phase described for tobacco, the necrosis passes up the petiole into the stem and the plant rapidly wilts and dies owing to the destruction of the vascular system (Pl. XV, fig. 4). Death nearly always ensues in young *N. glutinosa* plants inoculated with this virus.

Nicotiana langsdorffii

Local lesions are produced in this plant and small plants are frequently killed in a similar manner to *N. glutinosa*. A systemic necrotic disease usually develops in this species.

Tomato

The symptoms on tomato consist usually of a mottling only; the virus seldom produces the gross lesions on the stem which are typical of tomato streak virus 1.

Datura stramonium

This plant is frequently killed by a stem necrosis developing from local lesions on the inoculated leaves, a very similar symptom picture to that shown in *Nicotiana glutinosa* and the necrotic phase in tobacco (Pl. XV, fig. 3).

Hyoscyamus niger

Systemic infection with a pronounced mosaic mottle develops in *H. niger*. There is not much leaf deformation and local lesions may or may not be present.

Petunia (*garden variety*)

In petunia the virus produces no local lesions but systemic infection always ensues. There is a well-marked dark and light green mottling and the younger leaves are deformed in a characteristic manner. At the apices the leaf edges roll inwards towards each other while the base remains normal. This gives a scoop- or cup-like appearance to the leaves.

Potato (*var. Arran Victory*)

Local lesions only are produced on the inoculated leaves of this potato variety. There is no systemic invasion. The lesions are similar to those produced by inoculation with tobacco virus 1.

In many ways the symptoms induced by this virus in different hosts resemble those described by Ainsworth (1933) for tomato streak (the old tomato stripe), but there are differences.

The writer considers that this virus is probably a strain of tomato streak although it partakes of the characteristics both of tomato streak and tobacco virus 1. The virus seems to come midway between these two. It resembles tomato streak virus 1 in the stem necrosis it occasionally induces in tobacco and in the leaf mottling caused in that plant; it resembles tobacco virus 1 in the mottling it produces in tomato, usually without stem necrosis, but differs from it in the comparative absence of leaf distortion produced in tobacco. It appears to differ from both these viruses by the fact that it is usually fatal to young *Nicotiana glutinosa* plants.

It is doubtful how far the production of local lesions in tobacco (White Burley) is a trustworthy diagnostic character. It is no very uncommon thing to find that the same strain of streak virus may at one time give local lesions and at another fail to do so. Indeed the writer has had experience of this phenomenon resulting from inoculations with the same lot of streak virus inoculum on closely similar White Burley tobacco plants.

Infection by spraying

Duggar and Johnson (1933) have shown that it is possible to infect healthy tobacco plants by spraying them by means of an atomiser with a suspension of tobacco virus 1. These experiments have been repeated with the streak virus substituted for that used by Duggar and Johnson. A sand and pulp filtrate of undiluted virus sap was sprayed on to young tobacco seedlings by means of a De Vilbiss atomiser. In one such experiment, out of fifty plants of White Burley tobacco seedlings treated in this manner thirty-five became infected with the streak virus. A somewhat similar result has been obtained with the *X* virus on tobacco and the virus of tobacco necrosis on cowpeas (Smith and Bald, 1935). Since the percentage of infections obtained is far too high to be explained on a basis of accidental contamination, and since there is no reason to suppose that the plants were damaged or wounded in any way at the time of, or by the act of, spraying, it seems reasonable to conclude that the virus entered by the stomata, as suggested by Duggar and Johnson.

A yellow variant of the streak virus

The streak virus described above has been kept going for four years by periodic transfers in White Burley tobacco. Some months ago it was noticed that one of the infected plants had developed a small yellow spot. This spot of tissue was carefully cut out and inoculations made from it to young tobacco plants and the same process of selection practised at each passage. After about six serial transfers of this nature, in which the yellow tissue alone was used as a source of inoculum, a virus was isolated which produced a bright yellow

mottling (Pl. XVI, figs. 1, 2). As the transfers proceeded the number of yellow patches increased and the green mottling characteristic of the original strain became less.

The symptoms produced by the yellow strain on *Nicotiana glutinosa*, *N. langsdorffii* and *Datura stramonium* were of the necrotic type and were indistinguishable from the lesions produced by the green strain. In experiments where it was desired to distinguish between the two virus strains tobacco plants, White Burley variety, were always employed, since this plant immediately differentiates the two. Local yellow spots were also produced by the yellow strain on tomato and *Solanum nigrum*.

Comparisons in behaviour of the green and yellow strains

It has not been found possible to isolate the yellow strain of virus absolutely free of the green, although, as will be shown later, it is possible to accomplish the reverse process. Jensen (1933) has used successfully a needle-prick method to isolate yellow strains of tobacco mosaic, but this method did not succeed in the present case. The procedure consists in pricking with a sterile needle through a yellow spot into a healthy leaf held beneath. It was found that the percentage of successful transmissions of the yellow strain by this method was very low compared with the percentage of successful transmissions of the green strain. If the yellow virus was so transmitted, however, the green virus invariably accompanied it even though the needle was passed only through a yellow spot.

When the yellow strain is transmitted to White Burley tobacco, either by the needle-prick method or by ordinary methods of inoculation, yellow spots are always produced on the inoculated leaves. The subsequent behaviour of the two virus strains in an infected White Burley tobacco plant is of some interest. No matter how concentrated the yellow strain appeared to be in the initial inoculum the *final* symptom picture of the affected tobacco plant was always the same, *i.e.* a great preponderance of green virus with here and there a few yellow flecks. The only apparent result of increasing the concentration of yellow virus in the inoculum was to increase slightly the number of local yellow spots on the inoculated leaf. It is in the formation of these local yellow spots that the explanation of this behaviour seems to lie. While the yellow strain of virus is multiplying locally to form the spots, the green virus which usually produces no local symptoms passes on and becomes systemic in the plant. Now it is shown in a subsequent paragraph that the green strain confers upon the plant an immunity to infection with the yellow strain, consequently the latter would be unable to travel farther in a plant already systemically infected with the green strain. The yellow virus is therefore more or less inhibited from further development, although isolated yellow flecks may occur in the younger leaves. It must be supposed that these flecks represent small areas where the green virus was not systemic, and these areas would afford opportunity to the yellow strain to multiply. This supposition is further sup-

ported by the immunity tests (p. 456) with the two strains in which the yellow virus occasionally obtains entry at a few points when inoculated to plants already systemically infected with the green strain. Bawden (1934) has described a similar occurrence in immunity tests with potato virus *X*.

In carrying out the needle-prick method of inoculation the experiment has been made of passing the needle through the yellow spot directly into the midrib or large vein of the healthy leaf underlying it. When this has been done it has occasionally happened that the yellow virus has been introduced directly into the phloem and has been carried straight to the young leaves where it has produced large yellow blotches still, however, in company with the green strain. If the yellow virus is introduced directly into the midrib of a leaf in a tobacco plant already infected with the green strain no additional symptoms develop. These facts seems to support the foregoing suggestion that after initial infection the green strain is carried in the phloem ahead of the yellow strain and becomes systemic to the exclusion of the latter.

The behaviour of these two strains may with advantage be compared with that of the *X* and *Y* viruses in a mixture in the same variety of tobacco plant (Smith, 1931). After inoculation with this complex the *Y* virus moves off ahead of the *X* virus and can be obtained alone from the young leaves. Now since neither the *X* nor *Y* virus immunises against the other, the *X* virus is able to follow on and become equally systemic in the plant with the *Y* virus, the two producing together the necrotic disease characteristic of this double infection.

In addition to the interaction of rates of movement and immunity described above there can be little doubt that the green strain multiplies more rapidly than, and at the expense of, the yellow strain.

Other attempts to isolate the yellow strain by means of single lesions were also made (Jensen, 1933). An inoculum was prepared by cutting out the yellow patches from an infected White Burley tobacco plant and inoculations made at various dilutions to *Nicotiana glutinosa* and *Datura stramonium*. The resulting lesions were then cut out and inoculated singly to healthy White Burley seedlings. It was found that by far the greater number of lesions yielded the green strain only, while, if the yellow strain was obtained, the green strain accompanied it. It was exceedingly difficult to recover the yellow virus at all from *Datura stramonium*. These results also seem to point to a slower rate of multiplication on the part of the yellow strain and to emphasise its lower infectivity.

Separation of the green and yellow strains by ultrafiltration

Elford (1931) has recently developed a technique for producing collodion membranes of graded and uniform pore size. Through the kindness of Prof. D. Keilin opportunity has been afforded at the Molteno Institute for the preparation of these membranes and they have been used in some filtration experiments with the green and yellow strains of tomato streak. The virus sap

was extracted from White Burley tobacco plants infected with the two strains and used undiluted. In every experiment a greater quantity of yellow tissue than green was used in order to compensate as far as possible for the less infectious nature of the yellow strain and its apparently lower virus content. The virus sap was cleared of visible particles and prepared for filtration by passage of a sand and pulp filter; occasionally the sap was clarified by passage of a Kieselguhr bed. In each case before filtration the titre of the virus suspension was taken. It was found that while the green strain would easily stand a dilution of 1 : 10,000, the yellow strain would seldom give infection at 1 : 1000. This fact is of importance in view of the filtration results.

The data are given in Table I, and it will be seen that the filtration end-point differs for the two virus strains. The average pore diameter of the limiting membrane for the green strain appeared to be in the neighbourhood of 0·086 μ, while the yellow strain was held back by a membrane of approximately 0·16 μ average pore diameter. The green strain can therefore easily be separated from the yellow by ultrafiltration.

It seems probable that this difference in filterability between the two strains is largely a quantitative one and cannot be assigned to an actual difference in particle size. The low dilution end-point of the yellow strain favours this view.

Table I. *Filtration of a mixture of the green and yellow strains of tomato streak virus through graded collodion membranes*

Membrane thickness (mm.)	Average pore diameter of membrane (μ)	Controls sand and pulp filtrate	Experimental plants
0·12	0·43	± ± ± + + +	± ± ± + + +
0·16	0·31	± ± ± ± ± ±	± ± ± ± ± ±
0·16	0·16	± ± ± ± + +	± ± + + + +
0·165	0·16	± ± ± ± ± ±	+ + + + + +
0·195	0·10	± ± ± ± ± ±	+ + 0 0 0 0
0·20	0·098	± ± ± ± ± ±	+ + + + + +
0·20	0·098	± ± ± ± + +	+ + + + 0 0
0·23	0·086	± ± ± ± ± ±	+ + 0 0 0 0
0·23	0·086	± ± ± ± + +	0 0 0 0 0 0
0·23	0·086	± ± ± + + +	0 0 0 0 0 0
0·21	0·074	± ± ± ± ± ±	0 0 0 0 0 0
0·21	0·074	± ± ± ± + +	0 0 0 0 0 0
0·21	0·074	± ± ± ± ± ±	0 0 0 0 0 0

The *p*H of the virus sap varied between 5·6 and 6·2. Six controls and six experimental plants were inoculated in each case.

± = positive infection with green and yellow strains.

\+ = positive infection with green strain only.

0 = no infection.

Filtration pressure was 30 lb. per sq. in.; 5 c.c. were filtered in each case. All inoculations were made to White Burley tobacco.

Although the yellow strain as extracted from the plant will seldom stand a dilution of 1 : 1000, it has been experimentally concentrated on an impermeable membrane so as to give positive infections at a dilution of 1 : 10,000. Until the possibility of filtering the yellow strain in higher concentrations has been further explored, no attempt has been made to calculate the particle size of the respective virus strains from the foregoing data.

Immunity tests

The work of Thung (1931), Salaman (1933), Kunkel (1934) and Bawden (1934) has shown that systemic virus infection in a plant will preclude the entrance into that plant of another strain of the same virus or of another virus closely similar in character. This immunity depends on the prior establishment of one virus in the plant; if both viruses are inoculated simultaneously both become established. Previous experiments on these lines have indicated that this immunity holds good only for closely similar viruses and virus strains, and the present work affords further support for this view.

The first immunity tests were made with the yellow and green strains against each other. Tobacco plants, White Burley variety, systemically infected with the green strain, were inoculated with the yellow strain, while healthy plants of the same age were also inoculated with the yellow strain as controls. As shown in Pl. XVI, fig. 3, the yellow virus failed to enter the plants already systemically infected with the green strain, while the control plants developed the yellow spots characteristic of infection with the former virus. In some cases one or two odd spots of yellow developed on leaves carrying the green strain, in places where systemic invasion with the latter was possibly not complete. Conversely, the green strain failed to enter plants infected with the yellow strain, but no deduction can be drawn from this because, as previously pointed out, the green strain always accompanies the yellow and so would be already present in the test plant.

The next tests were made against two other strains of tomato streak, kindly supplied by Dr Ainsworth and Mr Bald, which produced local necrotic lesions on inoculation to White Burley tobacco. Here again these two strains failed to produce necrotic lesions on plants systemically infected with the green and yellow strains under study but did produce them on the healthy control plants. Similarly complete cross-immunity was found to exist between these two strains of tomato streak and the virus of tobacco mosaic (tobacco virus 1). The results afford additional evidence of the close relationship that seems to exist between these mosaic viruses.

Some further immunity tests were then made, using viruses of quite a different character. The first was the virus of tobacco necrosis (Smith and Bald, 1935), which produces only necrotic symptoms. This virus entered freely into tobacco leaves systemically infected with either strain of the streak virus. The converse was also true, but this would be expected in any case since the virus of tobacco necrosis is confined to the necrotic lesions it produces.

There was no protective action between potato virus *X* and the two strains of streak. The latter entered normally into young White Burley tobacco plants which were systemically infected with the *X* virus, producing a severely necrotic and usually lethal disease.

No cross-immunity could be demonstrated between the streak viruses and the viruses of tomato spotted wilt and tobacco ringspot (Wingard, 1928).

Effect of the yellow strain of virus on chlorophyll

The view has been put forward by Sheffield (1933) that the chlorotic spots in plants affected by the virus of yellow or aucuba mosaic of tomato are brought about by the inhibition by the virus of development of the plastic primordia and not by the destruction of mature plastids. It is difficult to apply this reasoning to the action on chlorophyll of the present yellow strain of virus. Local yellow spots develop on the inoculated leaves of White Burley tobacco within four or five days of inoculation (Pl. XVI, fig. 2). These inoculated leaves may be quite large and in some of the experiments were 9 or 10 in. in length, almost mature and already yellowing. In Pl. XVI, fig. 4, is shown one such leaf; this was 9 in. long and was one of the basal leaves of quite an old plant. Yet the yellow spots seen in the photograph commenced to appear four days after inoculation, although their subsequent development was somewhat slower than that of the spots in the younger leaf shown in Pl. XVI, fig. 2.

Since these yellow spots are produced in leaves which have practically ceased growing, it would seem that this virus has a definitely destructive action on the mature plastids rather than an action which is inhibitory of plastid formation.

DISCUSSION

The behaviour of the streak virus of tomato as described in this communication is further evidence of the variability of plant viruses. Not only is the green strain itself slightly different in its behaviour from most strains of tomato streak virus 1 but it is highly probable that the yellow strain has arisen as a mutation of it. It seems hardly likely that the yellow variant could have been present during the four years the green strain has been cultured without being seen, nor is it likely to be a contamination, as it is unlike any other virus at present under study at Cambridge. It has been compared with a yellow strain of tobacco virus 1, kindly sent to the writer by Dr Jensen, and there can be no doubt that the two are different as judged by their respective symptom pictures and behaviour on White Burley tobacco.

The fact that the virus of tomato streak together with the virus of tobacco mosaic can easily be transmitted to healthy susceptible plants merely by spraying them from an atomiser may in time lead to the discovery of a method of virus transmission hitherto unsuspected. It certainly seems necessary to consider the possible existence of a natural mode of transmission for some of the plant viruses other than by the agency of insect vectors or accidental contamination.

The immunity which is conferred upon a plant by one virus against another closely similar virus or virus strain is likely to prove a useful tool in the differentiation or grouping of plant viruses. It also involves some interesting implications in regard to the natural occurrence of virus complexes and complexes of virus strains. Firstly it seems necessary to assume that the component viruses of all virus complexes occurring in the field cannot immunise against each other and the complexes must therefore consist of viruses which are very

different in character. The *X* and *Y* viruses which frequently occur together in potato plants in the field are a case in point. Clearly, if the second virus of the complex is not of a different nature from the first, its entrance into the plant will be inhibited by the virus already there, *unless* it be assumed either that an insect vector exists which is able to carry both viruses at once and inject them simultaneously or that several insects carrying separate viruses feed on the same plant at the same moment.

The second point is the indirect evidence afforded by this immunity concerning the origin of plant virus strains. Here again it must be assumed that the various strains of tomato streak or tobacco mosaic can only be transmitted to healthy plants *en bloc* and not one at a time. While it may be admitted that this could happen easily enough in laboratory transfers by sap transfusion, it is a different matter so far as *naturally* infected plants in the field are concerned. Potato plants can frequently be found in the field naturally infected with the *X* virus, and from such a plant it is an easy matter to isolate two or more strains of the *X* virus. Here then, since one strain of *X* inhibits the entrance of another strain (Salaman, 1933), as suggested by Bawden, it seems necessary to assume either that the transmitting agent conveys all the *X* strains together, which is unlikely, or that one strain gives rise to others during its sojourn in the plant.

SUMMARY

The symptoms produced by a green strain of tomato streak virus 1 upon a variety of Solanaceous plants are described. A yellow variant of the streak virus which appears to have arisen during the culturing of the green strain is also described. This variant produces local yellow spots upon all inoculated leaves of White Burley tobacco.

Healthy tobacco plants have been infected with the green strain of the streak virus by spraying them with a virus suspension from an atomiser.

The behaviour of the green and yellow virus strains when co-existing in the tobacco plant is discussed.

Filtration through graded collodion membranes shows that the two strains have different filtration end-points and that therefore they can be separated by this means. The difference in filterability is considered to be a quantitative effect and cannot yet be assigned to difference in particle size.

Immunity tests have shown that a complete cross-immunity exists between the green and yellow strains of streak themselves, between them and two other strains of streak which produce local lesions and between the green and yellow strains and tobacco virus 1. No immunity was found between the streak viruses and those of tobacco necrosis, tobacco ringspot, tomato spotted wilt and potato virus *X*.

The yellow strain of streak produced local yellow spots on mature leaves of White Burley tobacco. This suggests that the chlorophyll is actually attacked by the virus, and that the formation of the chlorotic spots is not due merely to the inhibition of plastid formation by the virus.

ACKNOWLEDGMENTS

The writer has pleasure in acknowledging the assistance given by Mrs D'Oliveira and Mr J. P. Doncaster in the course of this work.

REFERENCES

AINSWORTH, G. C. (1933). An investigation of tomato virus diseases of the mosaic, "stripe", streak group. *Ann. appl. Biol.* **20**, 421–8.

AINSWORTH, G. C., BERKELEY, G. H. and CALDWELL, J. (1934). A comparison of English and Canadian tomato virus diseases. *Ibid.* **21**, 566–80.

BAWDEN, F. C. (1934). Studies on a virus causing foliar necrosis of the potato. *Proc. roy. Soc.* B, **116**, 375–95.

DUGGAR, B. M. and JOHNSON, B. (1933). Stomatal infection with the virus of typical tobacco mosaic. *Phytopathology*, **23**, 934–48.

ELFORD, W. J. (1931). A new series of graded collodion membranes. *Journ. Path. and Bact.* **34**, 505–21.

JENSEN, J. H. (1933). Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic. *Ibid.* **23**, 964–74.

KUNKEL, L. O. (1934). Studies on acquired immunity with tobacco and aucuba mosaics. *Ibid.* **24**, 437–66.

MCKINNEY, H. H. (1926). Virus mixtures that may not be detected in young tobacco plants. *Ibid.* **16**, 893.

—— (1931). Differentiation of viruses causing green and yellow mosaics of wheat. *Science*, **73**, 650–1.

MURPHY, P. A. and M'KAY, R. (1932). The compound nature of crinkle and its production by means of a mixture of viruses. *Sci. Proc. R. Dublin Soc.* **5**, 227–47.

PRICE, W. C. (1934). Isolation and study of some yellow strains of cucumber mosaic. *Phytopathology*, **24**, 743–61.

SALAMAN, R. N. (1933). Protective inoculation against a plant virus. *Nature*, Lond., **131**, 468.

SHEFFIELD, F. M. L. (1933). The development of assimilatory tissue in Solanaceous hosts infected with aucuba mosaic of tomato. *Ann. appl. Biol.* **20**, 57–69.

SMITH, KENNETH M. (1931). On the composite nature of certain potato virus diseases of the mosaic group. *Proc. roy. Soc.* B, **109**, 251–67.

—— (1933). The present status of plant virus research. *Biol. Rev.* **8**, 136–79.

SMITH, KENNETH M. and BALD, J. G. (1935). A description of a necrotic virus disease affecting tobacco and other plants. *Parasitology*, **27**, No. 2.

THUNG, T. H. (1931). Smetstof en plantencel bij enkele virusziekten van de tabaksplant. *Hand. ned. ind. natuurw. Congr.* **6**, 450–63.

WINGARD, S. A. (1928). Hosts and symptoms of ringspot: a virus disease of plants. *J. agric. Res.* **37**, No. 3.

EXPLANATION OF PLATES

PLATE XV

Fig. 1. Tobacco plant (var. White Burley) infected with tomato streak virus 1. Note the necrotic lesion on the stem.

Fig. 2. Tobacco plant (var. White Burley) killed by streak virus 1. The plant has finally collapsed owing to the destruction of the vascular system.

Fig. 3. Plant of *Datura stramonium* infected with streak virus 1. This plant collapsed in the same manner as the tobacco plant shown in Fig. 2.

Fig. 4. Young plant of *Nicotiana glutinosa* killed by a systemic necrosis similar to that which affects tobacco and *Datura*.

All the above plants are affected with the green strain of tomato streak virus 1.

PLATE XVI

Fig. 1. Young plant of tobacco (var. White Burley) infected with the yellow strain of tomato streak virus 1. The lower leaf has been inoculated, the upper one shows systemic infection only. Note the tendency to ring formation on the inoculated leaf.

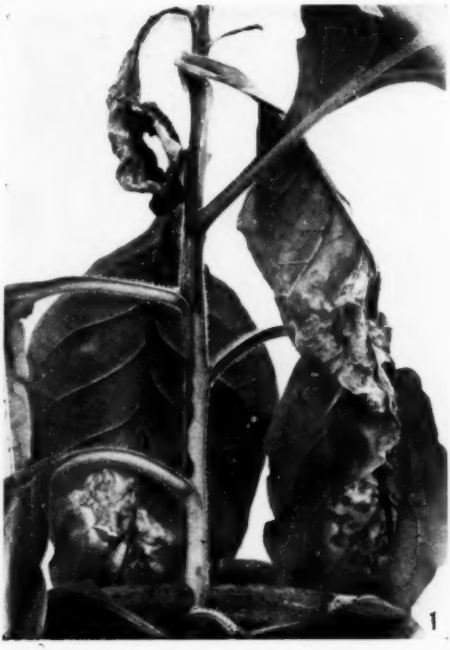
Fig. 2. Larger plant of tobacco (var. White Burley) infected with the yellow virus, inoculated leaves only showing symptoms. Note the presence of large numbers of yellow spots locally on the inoculated leaves.

Fig. 3. Two leaves of tobacco (var. White Burley) inoculated with the yellow streak virus; the leaf on the left was healthy, that on the right was already systemically infected with the green strain. Note that while the healthy leaf developed numerous yellow spots, the leaf already infected was protected against invasion by the yellow strain.

Fig. 4. This photograph shows the development of local yellow spots on a mature leaf of tobacco (var. White Burley). Note that the tip of the leaf is already yellowing. Length of leaf 9 in.

(Photographs by J. P. Doncaster)

(*MS. received for publication* 28. II. 1935.—Ed.)


1

2

3

1

2

3

4

UNIV. OF MICH.

ON THE PRESENCE OF WING BUDS IN THE PUPA OF APHANIPTERA

By M. SHARIF, D.Sc.

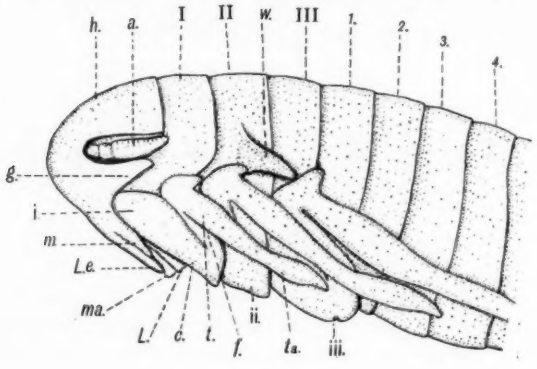
From the Molteno Institute of Biology and Parasitology, University of Cambridge

(With 2 Figures in the Text)

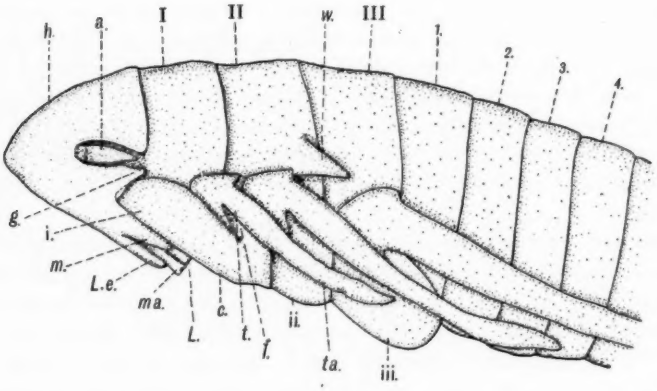
The question of the origin of the Aphaniptera has been, so far, considered obscure. It is for the supposed complete absence of wings in all stages that these insects have been thought by some authors to constitute a very sharply defined order having no close connection with any other group of insects (Imms, 1934, p. 688; Patton and Cragg, 1913, p. 434). This total absence of wings in an order of insects which has a complete metamorphosis would have given this group a very anomalous position in the insect world, as there is no holometabolous insect known which does not possess wings in any stage of its existence. For this reason Packard's conclusion (1894, p. 331) "Indeed, the three-fold division of the thorax referred to should have *a priori* forbidden the thought of rudimentary wings", and Patton and Cragg's statement (1913, p. 434) "They bear no recognizable traces of wings, and the structure of the thorax makes it very doubtful if they are descended from winged forms at all", can scarcely be accepted.

Some earlier workers on this group could not accept that fleas, which are holometabolous insects, are completely devoid of wings in any stage of their existence. They have, therefore, tried to recognise wings in the epimera of the metathorax of the adult flea (*vide* Jordan and Rothschild, 1908, p. 27; Rothschild, 1898, p. 534; Tiraboschi, 1904, p. 217). But the presence of wings on the metathorax only would again have given fleas a very unique position among the insects. Heymons (1899, p. 232), who examined the pupae and the larvae of *Pulex gallinae* Bouché [which is a synonym of *Ceratophyllus gallinae* (Schrank)], failed to find any trace of the imaginal buds of wings on the mesothorax and the metathorax, as is clear from the following statement of his: "Anzeichen von Imaginalscheiben im Meso- und Metathorax, die etwa als Flügelrudimente aufgefaßt werden könnten, sind weder bei der Larve noch bei der Puppe von *Pulex gallinae* vorhanden. Dieser Umstand spricht vielleicht dafür, daß der Verlust der Flugwerkzeuge bei den Siphonapteren bereits vor langer Zeit erfolgt sein muß, jedenfalls konnte ich ontogenetisch in dieser Hinsicht nichts nachweisen, was auf eine Beziehung der Flöhe zu jetzigen flügeltragenden Insecten hindeutet."

While examining the pupae of *Nosopsyllus fasciatus* (Bosc), I noticed a pair of appendages triangular in shape attached to the sides of the mesothorax and of about 0·18–0·20 mm. in length in specimens 2·47 mm. long. On account of



1



2

Fig. 1. *Nosopsyllus fasciatus:* anterior portion of the pupa showing a wing bud on the mesothorax, ×39.

Fig. 2. *Leptopsylla segnis:* anterior portion of the pupa showing a wing bud on the mesothorax, ×67.

a. antenna; *c.* coxa and trochanter; *f.* femur; *g.* gena; *h.* head; *i, ii, iii,* pro-, meso- and metathoracic legs; *I, II, III,* pro-, meso- and metathorax; *L.* labial palp; *L.e.* labrum-epipharynx; *m.* maxilla; *ma.* mandible; *t.* tibia; *ta.* tarsus; *w.* wing bud; 1, 2, 3, 4, first, second, third and fourth abdominal segments.

their shape and the position of their origin these structures cannot be anything but wing rudiments. In order to verify the presence of this structure in other species of fleas, I examined the pupa of *Xenopsylla cheopis* (Rothschild) but

found no trace of it, though the pupae of *Leptopsylla segnis* (Schöncherr) [=*Leptopsylla musculi* (Dugès)], which were placed at my disposal through the courtesy of Prof. P. A. Buxton, were found to possess these appendages. The following are the lengths of wing buds in the pupae of the last-named species, compared with the total lengths of the body:

Length of the wing bud	Total length of the pupa
mm.	mm.
0·1	1·7
0·12	1·9
0·15	2·0

Through the courtesy of Dr H. P. Bayon I was able to examine some living pupae of *Ceratophyllus gallinae* and found that the wing bud is as well developed in this species as it is in *Nosopsyllus fasciatus*. On account of the presence of this wing bud near the middle of the lateral surface of the thorax, it is difficult to detect its presence in mounted preparations, since it becomes pressed against the side of the thorax and its outline fades away. It is, however, distinctly visible as a definite structure in a living specimen, when examined without cover-slip, even under a low power of magnification. It is possible that Heymons only examined mounted preparations of the pupae of this species and thus failed to detect the presence of the wing bud.

On account of the presence of these appendages in the pupae of three species of two different families, viz. Leptopsyllidae and Ceratophyllidae, it is reasonable to presume that the ancestors of fleas had on the mesothorax, as in Diptera, functional organs of flight which are now only retained in a rudimentary form in the pupa. So far, I have not found any trace of wings in the adult flea. It is, therefore, legitimate to suppose that the wing buds of the pupa become completely absorbed in the adult, a presumption which is supported by our knowledge of some wingless Diptera. In *Belgica antarctica* Jacobs (Keilin, 1912, p. 725 and 1913, p. 226) the mesothoracic wings are long and fairly well-developed structures in the pupa, but are only small rudimentary structures in the adult. The explanation given by Keilin for this interesting phenomenon is: "La réduction de l'aile imaginale ne résulte pas de la réduction des disques imaginaux alaires chez la larve. C'est au moment de la métamorphose que se produit le véritable processus de réduction. L'aile imaginale, au lieu de proliférer à l'intérieur du sac chitineux constituant l'aile nymphale, se réduit au contraire, probablement par résorption, de telle sorte qu'elle devient beaucoup plus petite que celle de la nymphe (*fig.* II et VI)." I have noticed a similar reduction of cells, with recession from the inner wall of the chitinous sac constituting the pupal wing, in the pupa of the flea. It appears that the process of absorption of the cells constituting the pupal wing has been carried to a far greater extent in the flea than in *B. antarctica*, and as a result of that the wings have totally disappeared in the adult flea.

REFERENCES

HEYMONS, R. (1899). Die systematische Stellung der Puliciden. *Zool. Anz.* **22**, 223–40.

IMMS, A. D. (1934). *A General Textbook of Entomology*, pp. i–xii + 1–727. London.

JORDAN, K. and ROTHSCHILD, N. C. (1908). Revision of the Non-Combed Eyed Siphonaptera. *Parasitology*, **1**, 1–100, Pls. I–VII.

KEILIN, D. (1912). Zoologie.—Sur l'anatomie et le développement de *Belgica antarctica* Jacobs, Chironomide antarctique à ailes réduites. *C. R. Acad. Sci.*, Paris, **154**, 723–5.

—— (1913). Diptères. *Belgica antarctica* Jacobs. *Deuxième Expédition Antarctique Française* (1908–1910) *commandée par le Dr Jean Charcot*, pp. 217–31. Paris.

PACKARD, A. S. (1894). On the systematic position of the Siphonaptera, with notes on their structure. *Proc. Boston Soc. Nat. Hist.* **26**, 312–55.

PATTON, W. S. and CRAGG, F. W. (1913). *A Textbook of Medical Entomology*, pp. i–xxxiv + 1–764. Madras.

ROTHSCHILD, N. C. (1898). Contributions to the Knowledge of the Siphonaptera. *Novit. Zool.* **5**, 533–44, Pls. XV A, XVI, XVII.

TIRABOSCHI, C. (1904). Les Rats, les Souris et leurs parasites cutanés dans leurs rapports avec la propagation de la peste bubonique. *Arch. Parasit.*, Paris, **8**, 161–349.